MLSRB

MISSOURI LIFE SCIENCES RESEARCH BOARD

Comprehensive Progress Report FY2011

Report to the Governor and General Assembly

January 26, 2011

Missouri Life Sciences Research Trust Fund

Missouri Life Sciences Research Board Members

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1 Vacancy

In accordance with Section 196.1118, RSMo, every three years a "comprehensive report assessing the work and progress of the life sciences research program" shall be prepared and delivered to the governor and the general assembly. The purpose of this report is to provide a summary and impact of the research and commercialization projects approved and funded by the Missouri Life Sciences Research Board (MLSRB).

History and Mission

In 2003, House Bill 688 was enacted and created the MLSRB and the Missouri Life Sciences Research Trust Fund (Trust Fund). According to the statute, "moneys in the life sciences research trust fund shall be used strategically, in cooperation with other governmental and not-for-profit private entities, to enhance the capacity of the state of Missouri's ability to perform research to better serve the health and welfare of the residents of the state of Missouri as a center of life sciences research and development by building on the success of research institutions located in Missouri, creating in and attracting to Missouri new research and development institutions, commercializing the life sciences technologies developed by such institutions, and enhancing their capacity to carry out their respective missions."

The Trust Fund was created in order to enhance Missouri's position as a global leader in several important sectors of the life science economy – animal science, plant science, medical devices, biomaterials and composite research, diagnostics, nanotechnology related to drug development and delivery, clinical imaging, and information technology related to human health. This approach allows Missouri to build on its tremendous strengths in the agriculture sciences especially in plant biotechnology and animal health and nutrition, while also emphasizing the areas of medical devices, nanotechnology and other human health-related fields where Missouri has great potential. Ultimately, it is thought that placing resources where demonstrated strengths already exist will bring about the greatest results and the best return on the state's investment.

The mission of the MLSRB and the Trust Fund is to:

- Enhance research capacity in life sciences to better serve the health and welfare of Missouri citizens;
- Promote Missouri as a center of life science research and development by building on the success of existing Missouri research institutions;
- Create and attract new research and development institutions; and
- Transform research into commercial life science technology.

At the invitation of the MLSRB, the Missouri Technology Corporation (MTC) provides administration support for the Trust Fund. The MTC is a public-private partnership created by the Missouri General Assembly to promote entrepreneurship and foster the growth of new and emerging high-tech companies. MTC focuses on 21st Century bioscience industries that build on Missouri's rich history in agriculture. It is governed by a 15-member board of directors appointed by Missouri's Governor, Speaker of the House, and President Pro Tem of the Senate. The President of the University of Missouri System and the Director of the Department of Economic Development are *ex officio* members of the board.

While the MLSRB retains all decision-making authority, MTC plays an important role in ensuring that these dollars are invested wisely by managing the grant-making, monitoring, and compliance processes in a seamless, informative, and organized fashion. Key responsibilities performed by the MTC on behalf of the MLSRB include drafting the request for proposals (RFPs) for the research and commercialization projects, scientific peer review services, and the centers for excellence and providing administrative support to the MLSRB.

History of Funding

The promise of the life sciences in Missouri is impressive: to provide better health for citizens and animals, to create high quality, high tech jobs across the State, and to develop an economic engine to power future growth for our communities. The Missouri General Assembly authorized the Trust Fund in 2003 to help achieve this potential. The Trust Fund, established by section 196.1100, RSMo, receives twenty-five percent of all moneys received from the master tobacco settlement agreement and is subject to appropriation by the General Assembly.

For Fiscal Year 2008 the General Assembly and Governor appropriated $13.45 million to the Trust Fund in the areas of bioenergy, plant science and animal health and nutrition. After receiving 43 proposals the MLSRB awarded fourteen grants in the research and commercialization areas totaling $13.1 million.

In Fiscal Year 2009 the General Assembly and Governor approved $21 million to the Trust Fund ($13.4 million available for new grants with the remainder going to fund ongoing projects approved by the MLSRB). The language included an expansion into medical devices, biomaterials and composite research, diagnostics, nanotechnology related to drug development and delivery, clinical imaging and information technology related to human health. The MLSRB received 183 letter of intent proposals of which the MLSRB invited 66 to submit a full proposal. The MLSRB awarded eighteen grants totaling $13.1 million.

Pursuant to board policy and the appropriation, Centers for Excellence were required to focus their membership, expertise, and collaborations on all areas of research authorized by House Bill 2007. The following resolution was approved by the MLSRB:

> "*Recognizing that the Life Science Research Board has worked diligently to responsibly administer the monies entrusted to it by the Missouri General Assembly for fiscal year 2008, be it resolved that the Life Science Research Board strongly supports another year of funding for the Life Science Research Trust Fund and further specifies that it agrees with the recommendation to focus monies appropriated by the General Assembly exclusively on animal science, plant science, medical devices, biomaterials and composite research, nanotechnology related to drug development and delivery, clinical imaging, and information technology related to human health. Furthermore, the Life Science Research Board will not fund any human health research proposals outside of these scientific areas.*"

The General Assembly and Governor approved $13.3 million to the Trust Fund, with $13 million available for new grants in Fiscal Year 2010. Again, the language was specifically targeted for projects in the fields of animal science, plant science, medical devices, biomaterials and composite research, diagnostics, nanotechnology related to drug development and delivery, clinical imaging, or information technology related to human health. The MLSRB received 168 letter of intent proposals, 145 for research projects and 23 for commercialization projects. From the initial 168 proposals, the MLSRB invited 66 projects (53 research and 13 commercialization) to submit full proposals. The MLSRB was informed in late October 2009 that the Trust Fund was included in the state spending restriction; and therefore, **no grants were awarded in Fiscal Year 2010**.

No new funding was appropriated to the Trust Fund for Fiscal Year 2011.

Goals of the Life Sciences Research Trust Fund

The goal of the Trust Fund is to promote economic vitality in the State of Missouri by fostering innovative scientific research designed to improve the welfare of the State's citizens. Financial returns will leverage the dollars expended and potentially bring outside investment. Knowledge gains will enhance the reputation of Missouri as a global center of research, discovery, innovation, and commercialization. Scientific discovery will contribute to robust industry development and economic growth. Research will lead to innovations that will improve healthcare outcomes, efficiencies in delivery, and cost-effectiveness.

Research and commercialization will generate new scientific knowledge that will promote health and strengthen the State's reputation as a hub for innovation and biotechnology. These investments will enable innovative approaches to scientific problems, new collaborations among public and private institutions, and more rapid translation of discoveries from lab to market. Furthermore, this will assist in attracting new students and investigators to the region's research community.

It will do so in five important, measurable ways:

- ***Stimulate economic activity***. The Trust Fund grants will directly fund jobs and economic activity in Missouri, paying researcher salaries, supporting scientific staff, and promoting the purchase of goods and services within the state.

- ***Commercialization***. The Trust Fund grants will assist in furthering the commercialization of research discoveries, converting ideas into innovative goods and services for the marketplace, and fostering the creation and development of new companies. This commercialization process may produce invention disclosures, patent filings, intellectual property licensing, gap-funding grants, new company formation, and equity investments. Licensing of intellectual property will garner royalty income to institutions.

- ***Leveraging additional funds***. One of the most important returns on the State's investment in the Trust Fund will be its enabling role in helping institutions leverage additional research funding from non-state sources. Furthermore, additional funding may also be in the form of private investment in regards to commercialization activities that have been supported by the Trust Fund.

- ***Growing Research Capacity***. Research capacity is the volume of directed resources available to conduct research in a specific area of interest authorized by the General Assembly. Capacity can be increased in Missouri and retained, including personnel and equipment.

- ***Expanding Knowledge***. Publications in respected peer-reviewed journals and presentations to colleagues at major scientific gatherings are two common measures for the dissemination of new discoveries. To be accepted in these venues, articles and presentations must demonstrate sound science and represent a new contribution to the body of knowledge.

  The Trust Fund fosters new scientific knowledge focused on improvement in healthcare, agriculture, animal health, bioenergy, and biotechnology. To assist in achieving these benefits, the MLSRB has developed thorough standards and guidelines in making and managing its awards.

  The MLSRB has strived to deploy the Trust Fund to promote economic development within the State in three ways: (1) enabling new initiatives leading to the creation of jobs and additional economic activity; (2) attracting additional funding from non-state or industry resources; and (3) assisting in the commercialization of scientific innovation and the creation and development of new companies.

  The awarded grants are designed to foster projects with the leveraging potential to garner additional research funding or commercialization investment. Such projects might include:

  - Major initiatives to establish new research collaboration with increased competitiveness for non-state research dollars;
  - Projects to enable institutions to recruit prominent and renowned researchers or to acquire new equipment, both of which would have the potential to attract additional non-state funding, and increase capacity for additional research and economic growth; and
  - Projects where federal and industry funding is more difficult, especially in the cases where a successful innovation could lead to a major opportunity for future funding. Some grants provide "gap funding" for projects, bridging the divide between academic research discoveries and commercialization of products through the development of proof-of-concept activities or to develop a commercial prototype.

Project Proposal Criteria
Evaluations of the project proposals are based on two broad criteria:

1. **Scientific and technical quality of the proposed activity.** Proposals must address an important and relevant question(s) related to the specific research area(s) of interest to the MLSRB. The proposed project must exhibit innovation, scientific rigor and originality. The following factors will be considered in determining the project proposal's scientific and technical quality:

   - Degree of Innovation.

   - Expertise and research experience of the Principal Investigator, Co-Principal Investigators and collaborating investigators.

   - Quality and degree of collaboration(s) planned by the collaborating institutions and among the individual participants in the proposed activity and how those interactions will foster more rapid and higher quality progress toward goals of the proposed activity.

- Inventory of any specialized facilities, equipment and/or other resources required for the proposed activity indicating if they are currently available or being sought for performance of the proposed activity including location and availability of access and how the requested resources are key to the collaborative research effort in enabling both high priority research and research collaboration.

- Appropriate management of the proposed activity.

- Feasibility of the scope of work proposed for the period of funding.

- Appropriateness of the proposed budget with regard to the scope of work.

2. **Potential Impact of the Proposed Activity.** Proposals must exhibit the potential to provide a significant beneficial impact(s) to furthering the research and development capacity of the State of Missouri, job creation and the general health and welfare of the citizens of the state. The following factors will be considered in determining the proposed activity's potential impact:

    - Ability of the proposed activity to leverage additional funds from non-state sources (e.g. federal, foundation and private funding in the future to further support the specific research and/or commercialization activities.

    - Ability of the proposed activity to facilitate and promote the commercialization of discoveries and innovations that arise from research and development in the state. What are the timeline, scale, and scope of the commercialization opportunities?

    - Impact of the proposed activity on the field of research.

    - Alignment of the proposed activity with the state's strategic economic development and research priorities.

    - Potential contribution to the health and quality of life of the people of Missouri in the intermediate and/or longer term.

Review and Evaluation Process

Each proposal is evaluated and ranked by a Center for Excellence (CFE) which must be established within a geographical area specified in section 196.1106, RSMo. The CFE must be comprised of a consortium of public and private not-for-profit academic, research, or health care institutions or organizations that have collectively at least fifteen million dollars in annual research expenditures in the life sciences, including a collective minimum of two million dollars in basic research in life sciences.

For organizing purposes, each CFE is required to nominate a chairman and functional board of directors representative of the consortium of public and private not-for-profit academic, research, or health care institutions or organizations associated with their CFE with a focus on agriculture research and commercialization. Each CFE for life sciences research is required to appoint a screening committee. The CFE, through their screening committees, reviews, prioritizes, and forwards to the MLSRB the proposed research and commercialization initiatives for their funding consideration. Members of each screening committee are required to be generally familiar with the life sciences and current trends and developments with either technical or scientific expertise in the life sciences with an understanding of life sciences and with an understanding of the application of the results of life sciences research. No member of a screening committee may be employed by any public or private entity eligible to receive financial support from the Trust Fund.

The MLSRB views the regional and statewide CFE as virtual organizations, whose purpose is to think strategically about the life science research and commercialization initiatives important to their specific region, but also how these regional initiatives strengthen the state of Missouri's ability to compete on a larger regional and national scale. The CFEs were asked to develop the strongest possible proposals within their regions, looking to collaborate among other regions to enhance and strengthen the statewide base of research and development assets wherever possible.

Almost simultaneously, each proposal endures a rigorous Scientific Peer Review where it is reviewed and evaluated by a panel of experts based on the scientific and technical merit of the project.

The Scientific Peer Review firm must meet the following qualifications:

1. Experience in advising technical grant programs sponsoring projects within the life sciences;
2. Intimate knowledge of best practices in scientific peer review;
3. Significant experience and knowledge of issues pertaining to competitive grant programs funded by state government, including conflict of interest avoidance and public rights and access to information; and
4. Experience working with independent Web-based peer review systems.

During the Fiscal Year 2008 process, LYTMOS Group, LLC, located in Lee's Summit, Missouri was selected as the scientific peer review firm. LYTMOS demonstrated considerable experience performing similar peer reviews in the states of Florida, Pennsylvania, Maryland, and Indiana, as well as in the Kansas City area.

In Fiscal Year 2009 the peer review contract was awarded to the American Association for the Advancement of Science (AAAS) in Washington, D.C. Since 1996, AAAS has assembled and led carefully tailored teams to provide expert review and programmatic guidance on over 180 projects throughout the United States.

In Fiscal Year 2010, the MLSRB again selected LYTMOS Group, LLC as the scientific peer review firm. The scientific peer review was completed in November 2009.

Grant Awards

Within the limits of available funds, awards are made to applicants whose proposals are judged most meritorious under the evaluation criteria and procedures defined by the MLSRB. Using the CFE priorities and the Scientific Peer Review evaluations as a guide, the MLSRB determines which proposals will be funded, and any conditions that might pertain to the award of funds to each selected project.

Impact of Missouri Life Sciences Research and Commercialization Grants

Funded Fiscal Year	Life Sciences Research Trust Fund Grant Awards	Additional Funding Attracted	Matching/ In-Kind Funds	Number of Papers Published by Researchers	Number of Patents/ Disclosures Submitted
FY2008	**$13,100,000**	**$24,191,536**	**$2,098,754**	**52**	**9**
FY2009	**$13,147,690**	**$13,706,599**	**$882,031**	**43**	**3**
Total	**$26,247,690**	**$37,898,135**	**$2,980,785**	**95**	**12**

Source: Impact is self-reported by grantees.

Missouri Life Sciences Research Board
List of Meetings

2010	**February 4, 2010**
	April 8, 2010
	July 1, 2010
	November 5, 2010
	November 22, 2010
2009	**April 15, 2009**
	May 26, 2009
	July 1, 2009
	August 14, 2009
	November 2, 2009
	December 18, 2009
2008	**February 11, 2008**
	March 26, 2008
	June 3, 2008
	July 9, 2008
	July 23, 2008
	September 22, 2008
	October 3, 2008
	November 24, 2008
	December 19, 2008

FY2010 Life Sciences Research Trust Fund Grant Summary

***No grants were awarded in FY2010.**

Total Grant Funding Available in FY2010: $13,000,000

Research Funds Available in FY2010: $10,400,000
Commercialization Funds Available in FY2010: $ 2,600,000

Letter of Intent Proposals Received

Centers for Excellence	Requested Research Funds	# of Research Projects	Requested Commercialization Funds	# of Commercialization Projects
Kansas City	$19,136,466.00	27	$1,737,000.00	3
Springfield	$7,393,189.00	13	$809,000.00	2
St. Louis	$23,283,359.10	35	$3,920,663.00	6
Statewide	$58,775,852.76	70	$6,113,824.50	12
Subtotals	**$108,588,866.86**	**145**	**$12,580,487.50**	**23**

Full Proposals Received --Research

Centers for Excellence	Requested Research Funds	# of Requested Projects
Kansas City	$5,579,163.00	9
Springfield	$2,512,312.00	6
St. Louis	$8,520,941.00	12
Statewide	$27,574,577.38	26
Subtotals	**$44,186,993.38**	**53**

Full Proposals Received --Commercialization

Centers for Excellence	Requested Commercialization Funds	# of Requested Projects
Kansas City	$1,737,000.00	3
Springfield	$809,000.00	2
St. Louis	$1,090,663.00	3
Statewide	$3,249,496.00	5
Subtotals	**$6,886,159.00**	**13**

FY2009 Life Sciences Research Trust Fund Grant Summary

Total Grant Funding Available in FY2009: $13,100,000

Research Funds Available in FY2009: $10,500,000
Commercialization Funds Available in FY2009: $ 2,600,000

Letter of Intent Proposals Received --Research

Centers for Excellence	Requested Research Funds	# of Research Projects	Requested Commercialization Funds	# of Commercialization Projects
Kansas City	$26,680,047.00	43	$3,122,950.00	5
Springfield	$7,036,309.00	15	$1,197,600.00	2
St. Louis	$11,444,758.88	13	$4,136,000.00	5
Statewide	$67,547,438.21	88	$6,968,294.75	12
Subtotals	**$112,708,553.09**	**159**	**$15,424,844.75**	**24**

Full Proposals Received --Research

Centers For Excellence	Requested Funds	# of Requested Projects	Awarded Funds	# of Projects Awarded	% of Available Funds Awarded
Kansas City	$11,134,293.00	16	$2,432,153.00	4	23%
Springfield	$4,979,981.00	8	$825,000.00	1	8%
St. Louis	$9,177,498.20	8	$3,270,302.00	4	31%
Statewide	$29,063,772.67	27	$4,063,773.00	5	38%
Subtotals	**$54,355,544.87**	**59**	**$10,547,690.00**	**14**	**100%**

Full Proposals Received --Commercialization

Centers For Excellence	Requested Funds	# of Requested Projects	Awarded Funds	# of Projects Awarded	% of Available Funds Awarded
Kansas City	$0	0	$0	0	0%
Springfield	$574,450	1	$574,450	1	22%
St. Louis	$520,000	1	$520,000	1	20%
Statewide	$3,262,739	5	$1,505,550	2	55%
Subtotals	**$4,357,189**	**7**	**$2,600,000**	**4**	**100%**

FY2009 Funding Summary - Research

Grant Number	Project Title	Principal Investigator	PI Institution	Grant Category	Funds Awarded
09-1016	Acquisition of a Confocal Laser Scanning Microscope to Enhance the Research Capabilities of University of Missouri at St. Louis	Dr. Xuemin (Sam) Wang	University of Missouri-St. Louis	Equipment	$281,745
09-1018	Derivation of Induced Pluripotent Cells from the Pig	Dr. Toshihiko Ezashi	University of Missouri	Project Proposal	$180,000
09-1019	Workforce Development and Business Incubation: Animal Health and Nutrition Infrastructure in Missouri	Dr. Gary Clapp	Missouri Western State	Equipment	$285,000
09-1053	New Medical Materials, Devices, and Instrumentation at the Jordan Valley Innovation Center	Dr. Ryan Geidd	Jordan Valley Innovation Center/ Missouri State University	Centers or Institute	$825,000
09-1055	Pseudospark Pulsed Plasma X-ray Generation for Portable Medical Devices	Dr. Joshua Rovey	Missouri University of Science & Tech	Project	$164,268
09-1065	Informatics Research Core Facility	Dr. Mark McIntosh	University of Missouri	Project	$1,302,217
09-1076	St. Louis Institute for Nanomedicine	Dr. Samuel Wickline	Washington University	Centers or Institute	$1,500,000
09-1078	Computational simulation of canine biomechanically induced unicompartmental osteoarthritis: a concurrent multiscale approach	Dr. Trent Guess	University of Missouri-Kansas City	Project	$556,957
09-1101	UMKC Center of Excellence in Mineralized Tissues	Dr. Lynda Bonewald	University of Missouri-Kansas City	Centers or Institute	$1,050,196
09-1105	Acquisition of Metabolomics Platform for Metabolic Engineering	Dr. Leslie Hicks	Danforth Plant Science Center	Equipment	$894,993
09-1106	Drought Simulators Critical to Translational Research in Plant Science	Dr. Felix Fritschi	University of Missouri	Equipment	$1,558,125
09-1117	Advanced Cardiovascular Stent incorporated with Nitric Oxide Delivery System	Dr. Chi Lee	University of Missouri-Kansas City	Project	$540,000
09-1128	Targeting Plasminogen Activator Inhibitor-1 to Inhibit Restenosis	Dr. William Fay	University of Missouri	Project	$815,625
09-1148	Optimization of Camelina as a nonfood production platform of value-added biotechnology products.	Dr. Eliot Herman	Danforth Plant Science Center	Centers or Institute	$593,564
				TOTAL	**$10,547,690**

FY2009 Research Project Summaries

Project #:	09-1016
Project Title:	Acquisition of a Confocal Laser Scanning Microscope to Enhance the Research Capabilities of University of Missouri at St. Louis
Award Amount:	$281,745
Center for Excellence:	St. Louis
Lead Investigator:	Dr. Xuemin (Sam) Wang, University of Missouri-St. Louis
Collaborators:	Dr. Elizabeth A. Kellogg, University of Missouri-St. Louis; Dr. Colin MacDiarmid, University of Missouri-St. Louis; Dr .Lisa Schechter, University of Missouri-St. Louis; Dr. Teresa Thiel, University of Missouri-St. Louis; Dr. Amy Zanne, University of Missouri-St. Louis; Dr. Bethany K. Zolman, University of Missouri-St. Louis; Dr. Sonya Bahar, University of Missouri-St. Louis; Dr. James K. Bashkin, University of Missouri-St. Louis; Dr. Cindy Dupureur, University of Missouri-St. Louis; and Dr. Jingyue (Jimmy) Liu, University of Missouri-St. Louis

Summary:
This grant sought funds for a confocal laser scanning microscope (LSM) to improve the research capabilities of University of Missouri-St. Louis (UMSL) in areas important to plant science, bioenergy, medical devices, and biomaterials. LSM allows the user to peer deep into cells and tissues and to see internal cellular structures in astoundingly sharp detail. At the same time, preparation of materials to be observed requires minimal time; for many purposes, large pieces of plant or animal tissue can be placed under the microscope and internal structures can be observed directly. LSM has become the industry standard for microscopy; because it is both highly sensitive and time-efficient, it is a critical tool for biological and biomaterial research. With the development of new research programs and hiring of promising new faculty in the science departments at UMSL, the need for a state-of-the-art LSM is becoming necessary and urgent. However, there is no LSM system available on the UMSL campus. The acquisition of a Carl Zeiss LSM 710 will greatly enhance and expand the existing and future research efforts in many areas of biological and biomaterial research. The LSM system will be a versatile instrument that accommodates the diverse research needs in the departments of biology, physics, and chemistry and the Center for Nanoscience.

The availability of an LSM 710 system will improve the research capabilities and faculty's competitiveness for extramural research funding and for translational research. One application of the LSM is to enhance the research activities in several labs in searching for more efficient ways to capture and convert solar energy to produce bioenergy. The instrument and research activities will provide excellent opportunities to integrate frontier research with education to train students and scientists in research areas important to plant science, bioenergy, medical devices, and nanotechnology.

Update:
A Carl Zeiss LSM 700 confocal microscope imaging system has been purchased and installed in the Center for Nanoscience. Accessories required for imaging acquisition and data analysis have also been purchased and installed. A number of students and faculty have been trained in using the state-of-the-art imaging instrument. Research results are being collected using the instrument. The availability of the confocal imaging system has filled a critical need on campus' research capabilities and education.

Project #:	09-1018
Project Title:	Derivation of Induced Pluripotent Cells from the Pig
Award Amount:	$180,000
Center for Excellence:	Statewide
Lead Investigator:	Dr. Toshihiko Ezashi, University of Missouri
Collaborators:	Dr. Randall Prather, University of Missouri

Summary:
The ability to copy valuable animals in such a way that their merits, for e.g. milk production, semen quality, rate of gain, disease resistance, are maintained in the progeny of the original animal continues to be a problematic and inefficient process. One way to make progress in this area is to create pluripotent cell lines from the skin or some other accessible tissue of the animal, a process that could be achieved without killing or harming the donor animal, e.g. the valuable pig or cow. Their goal is to express a suite of special genes in these cells in such a way that they become reprogrammed and completely undifferentiated.

Such cells are known as induced pluripotent cells and can contribute to all the tissues of the body. However, they can be derived from a relatively small biopsy of ordinary tissues of the adult animal, without resorting to manipulation or loss of a live embryo. They seem to have achieved this end with porcine fibroblasts by expressing a combination of just four genes in addition to supplementing with a drug and a specialized growth factor. If pluripotency can be established in these cells, they will have great value for exact copying the pig by Dr. Randall Prather at the National Swine Center at the University of Missouri. The induced pluripotent cells are likely to provide greater efficiency than established procedures, and should likely mitigate the abnormalities and deaths that are common to animals cloned from differentiated cells, which appear not to be programmed correctly.

There are three aims to the proposal described here, plus a future long term aim for which funding is not presently being sought. Aim 1 is to create several induced pluripotent cell lines and to establish optimal culture conditions and freeze storage conditions for these cells. The second is to demonstrate that the cells are pluripotent according to standard criteria. Such cells, for example, should continue to grow indefinitely without senescing and have a stable chromosome complement throughout this time. They are expected to express genes typical of undifferentiated rather than differentiated cells, yet be capable, given an appropriate stimulus, to differentiate into a multitude of different tissue types, e.g. to nerve cells or liver cell. Aim 3, will compare different combinations of genes for re-programming differentiated pig somatic cells and determine which one is optimal. A final, longer term goal is to collaborate with Dr. Prather to show that these cells can be used to create pigs that are exact copies of the animal that contributed the cells in the first place. The results obtained in the grant are likely to lead to commercial development and allow the investigators to gain large scale federal and industrial funding and additional research stemming from the project.

Update:
Successful establishment of pluripotent embryonic stem cells from ungulates, especially pigs, is an important but challenging endeavor. The pig is an attractive species for creating pluripotent cell lines because such cells will provide a stable source of valuable agricultural production traits and be essential to establish the gene knock-in/knock-out that allows gene alteration in the species. It is anticipated such cells will avoid the inefficiencies and problems arising from somatic cell nuclear transfer, where the vast majority of cloned offspring die or are developmentally abnormal. Finally, the pluripotent stem cells from pig will be a useful model for studying human pathologies due to similarities in organ size, immunology and whole animal physiology between the two species. We have derived induced pluripotent stem cells (iPSC) from pig fibroblasts by means of retroviral transduction of the same four reprogramming genes (*OCT4, SOX2, KLF4,* and *c-MYC)* and published the results last summer (Ezashi,

Telugu et al. 2009). After our paper was published, we were approached by groups at UCLA, Baylor, Harvard, Stanford and others wanting to acquire the cells for studying their differentiation to cardiomyocytes.

Statement of Results:

Porcine iPSC provides great promise in agricultural and biomedical research. Therefore, the derivation of PiPSC we have accomplished with this grant is a significant milestone in the field. In summary, we have had success in generating large numbers of pluripotent cell types from the pig and are now confident we have accomplished the aims outlined in the proposal.

Ezashi, T., B. P. Telugu, et al. (2009). "Derivation of induced pluripotent stem cells from pig somatic cells." Proc Natl Acad Sci U S A **106**(27): 10993-8.
Yu, J., K. Hu, et al. (2009). "Human induced pluripotent stem cells free of vector and transgene sequences." Science **324**(5928): 797-801.

Project #:	09-1019
Project Title:	Workforce Development and Business Incubation: Animal Health and Nutrition Infrastructure in Missouri
Award Amount:	$285,000
Center for Excellence:	Kansas City
Lead Investigator:	Dr. Gary Clapp, Institute for Industrial and Applied Life Sciences
Collaborators:	Dr. Benjamin Caldwell, Missouri Western State University and Dr. Bern Eichenmueller, Boehringer Inghelheim Vetmedica

Summary:

The purpose of this project is to enhance the education and training of scientists and engineers in order to prepare them for careers in the Animal Health and Nutrition industries in Northern and Western Missouri. Current academic training does not satisfy the specific industrial need. The projected growth in the life science industry will only exacerbate the problem. The grant will be used to enhance training and educational activities being conducted at the Kit Bond Science and Technology Incubator through additional build out of a laboratory set to function under the current Good Manufacturing Practices (cGMPs) and Good Laboratory Practices (GLPs) regulations. Specifically, the funding will be used to build and operate a small functional clean room and to purchase additional scientific equipment and casework in support of this resource. The result will create a clean room in an environment that will not only simulate the environment where graduates will work, but could be used as a setting where incubator clients perform early hand filling and/or finish operations. The rationale for presenting this proposal lies in the cost of operations of a clean room. The cost to operate and/or access a clean room makes this resource difficult to afford and prohibitive for small and start-up firms.

The Institute for Industrial and Applied Life Sciences (IIALS) is a public-private partnership operating as a 501(c) 3 not for profit organization. The stakeholders in the IIALS include Missouri Western State University, Boehringer Ingleheim Vetmedica Inc, IVX Animal Health, AgriLabs, Clipper Distributing, Nestle Purina PTC, Heartland Health, the City of St. Joseph, Buchanan County, the St. Joseph Area Chamber of Commerce, The Wes Remington Family, The Bradley Family Trust and the Messick Trust. The IIALS's mission is to promote applied life science activities in the Northern and Western regions of Missouri, inclusive of Kansas City. The IIALS further defines its mission, vision and goals into three primary areas of emphasis: Workforce Development, Economic Development, and Advocacy for Animal Health and Nutrition.

IIALS also operates the Kit Bond Science and Technology Incubator and the Missouri Innovation Center of St. Joseph. The IIALS has the advantageous position of being able to offer access to these services and equipment needed by entrepreneurs as they begin to grow and develop their commercial products and ideas for the Animal Health Corridor.

Photo courtesy of IIALS

Update:

The IIALS completed construction of a Clean Room as part of the Missouri Life Science Trust Fund grant for Workforce Development Business Incubation: Animal Health and Nutrition Infrastructure in Missouri. A clean room and associated equipment were purchased, constructed, and installed in the MWSU Incubator. Validation of the function has been completed by Accutech. Dr. Gary Clapp and Sara Hagen managed the project and donated their time in-kind to make this project a reality.

Training activities have been initiated as part of the courses in workforce development at this location. Multiple courses were run this past year in concert/combination with the WIRED grant. A course entitled "Introduction to BioManufacturing" was developed through the WIRED grant and offered three times in our facility. A total of 26 students worked through this program and all graduated with certificates in BioManufacturing. The three courses grossed $58,000 in course fees for the region. The IIALS and its partners were presented an award by Governor Nixon at the KC Economic Development Summit for Innovative Workforce Development as a consequence of these courses.

Regional Professional Development Center (RPDC): The IIALS also ran several one day courses for the benefit of the regional high school and middle school Math, Science and Engineering teachers and their students. A total of 5 different groups of 8 or less were processed through the facility giving at least 40 training events and experiences during FY 2010. These training events also benefitted from the clean room and the equipment used to support clean room activities.

IIALS now plans to market the use of the clean room for outside companies. Activities inside the STI are already offered to clients of the IIALS. Access to the clean room and equipment is granted to firms that are occupants of the STI, as well as, virtual and emerging firms that may need access to specialized equipment and specialized training. Access to these resources is managed by the IIALS as part of its function as the operating entity for MWSU and the Innovation Center.

Statement of Results:
As a result of this grant, another valuable resource is available and can be offered to new and emerging firms in our region. The additional infrastructure will continue to help grow our economy and competent workforce.

Project #:	09-1053
Project Title:	New Medical Materials, Devices, and Instrumentation at the Jordan Valley Innovation Center
Award Amount:	$825,000
Center for Excellence:	Springfield
Lead Investigator:	Mr. Allen Kunkel, Missouri State University
Collaborators:	Dr. Paul Durham and Dr. Matthew Curry, Missouri State University

Summary:
The mission of the Jordan Valley Innovation Center (JVIC) is to improve the translation of research from the laboratory to the end user by providing an environment for entrepreneurship to flourish among scientists through intellectual property incentives. In the life sciences, corporate affiliates (including St. Johns Health Systems) help provide the background business information needed to drive high-risk high-reward research toward application. JVIC life science research focuses on medical materials, devices and instrumentation in both the pure and applied research venues.

Located in Springfield on the downtown campus of Missouri State University, JVIC has created a unique environment where corporate research scientists can work side by side with JVIC faculty, staff and students on externally funded programs. JVIC uses its seven senior corporate affiliates, three from within Missouri and four headquartered outside Missouri, to form a caucus that serves as a research and operational advisory board. This Senior Corporate Affiliate Caucus (SCrA) helps guide the research mission of JVIC and its intellectual property (IP), expansion, infrastructure and shared equipment policies. JVIC and its sub-centers operate as a non-profit institute where high-risk/reward research can be pursued without fear of losing commercial IP or patent rights. In addition, JVIC has a close relationship to Springfield Innovation, Inc., a Missouri Innovation Center, which provides further unique opportunities for the commercialization of research programs.

JVIC includes a 75,000 sq. ft. facility called the Roy Blunt JVIC building that includes 4,000 sq. ft. of Class 10, 100, and 1000 clean rooms that specialize in materials synthesis and nano/micro device fabrication. JVIC also houses medical instrument prototyping, medical materials, and microscopy laboratories. In addition to equipping these laboratories, JVIC has purchased over $20 million in state of the art materials synthesis and analysis equipment that is utilized by both the corporate affiliate scientists and the JVIC research staff. External research project income has totaled to over $35 million since FY03, not including facility renovation grants.

Medical research at JVIC is roughly divided among the physical length scales inherent in the project. At the smallest length scales JVIC is developing well known FDA approved materials systems into morphologies and microstructures that can improve long and short term in-vivo performance. At somewhat longer length scales, in-vitro experiments are performed to develop materials integrated with devices to make passive components active or "smart." At larger scales, fully integrated instrumentation compatible with existing signals and systems is being built to drive or provide adaptation to existing instruments or facilities. Finally, at the largest length scales, JVIC has specialized super computer and artificial intelligence facilities including a unique 0.46 TFOP (trillion floating point operations per sec) CRAY computer capable of developing models for ultra high speed analysis of vast arrays of medical related information.

Photo courtesy of JVIC.

Update:
The funding from the MLSRB grant has further developed the infrastructure and capacity of the JVIC. This investment has helped support several existing projects and will support future research projects in the area of life sciences. JVIC is comprised of two University research centers – Center for Biomedical and Life Sciences (CBLS) and Center for Applied Science and Engineering (CASE). These centers work in collaboration to support the MLSRB funding.

This funding has made a significant impact on the center's performance and capabilities. Although it is difficult to attribute this funding to directly attracting additional research dollars, it has allowed JVIC to support several existing projects by providing capacity and allowed JVIC to support strategic projects and technologies. This project will also allow the center to support long-term research activities that will benefit the region and state of Missouri. Future projects will include research in the areas of saliva diagnostics and natural products. Also, the project will explore additional research with developing collagen from the use of agricultural waste products. This type of investment will bring additional private research dollars to the University. JVIC is also working to develop an Advanced Materials Processing Laboratory that will provide additional capabilities in 3D composites.

Because of MLSRB's investment to build capacity for JVIC as an institute/center, JVIC has become the anchor of a broader vision and development in the Springfield region. IDEA Commons is Missouri State University's vision for an urban research park. This vision is widely supported by all the institutions in the community and will have tremendous economic impact on the University's research and commercialization opportunities.

Project #:	09-1055
Project Title:	Pseudospark Pulsed Plasma X-ray Generation for Portable Medical Devices
Award Amount:	$164,268
Center for Excellence:	Statewide
Lead Investigator:	Dr. Josh Rovey, Missouri University Science and Technology
Collaborators:	Dr. Scott Kovaleski, University of Missouri

Summary:
Current medical x-ray devices are large and rely on a high-voltage, high-current electron beam impinging onto a target to emit x-rays (bremstrahlung "braking" radiation). Further, the target must rotate at high speeds to dissipate the power of the beam. In the future, a small, portable x-ray device is envisioned that can be used on-site, at an accident, on the battlefield, in a clinic, or in the operating room. One potential technology that may achieve this goal is pulsed plasma based electron sources. In these devices, the high-energy electron beam is not space-charge limited, so higher currents at lower voltage and lower power can be obtained.

A pseudospark relies on pulsed current from a stored capacitor to generate a plasma discharge that emits a high-energy, high-current electron beam. Direction of this electron beam onto a target is known to produce EUV and soft x-ray emissions. With the proper scaling of the device geometry and pulse forming network, the pseudospark may also produce hard x-ray emissions that are suitable for medical applications. The benefit of this device is that it has a space-charge neutralized beam that decreases the required power for a given beam voltage (P~V2, as opposed to P~V5/2 for traditional space-charge limited x-ray sources). Since less power is required, the power supply electronics and thermal constraints are relaxed and a smaller device becomes possible.

The project consists of a two year study to investigate pseudospark plasma discharges for x-ray generation in a portable medical device. The overall objective is to demonstrate pseudospark xray production with the energy and quantity necessary for medical applications. They will combine modeling simulations and experimental testing to complete this objective in three main phases.

The expected outcome is a set of design criteria (geometry and pulse forming network) for developing a small, portable medical x-ray device that uses a plasma-based pseudospark for xray generation. First, an electron beam investigation will be completed. The initial pseudospark device will be designed based on previous results from a 100 kV device. However, this design will be modified and adjusted based on electron beam propagation and pseudospark operation modeling. Results from this phase will quantify the pseudospark-produced electron beam parameters, such as current, current density, and charge transfer per pulse. The optimum devices from this phase will be selected for x-ray measurements in phase 2.

Phase 2 will focus on x-ray measurements by placing a tungsten target downstream of the pseudospark device. Si PIN and CdTe diode detectors will be positioned to measure the x-ray spectrum. These results can be used to determine the exposure, absorbed dose, and other relevant metrics that will be compared with traditional x-ray tubes. In phase 3, the x-ray measurements will be used to determine which pseudospark devices are viable for medical applications. Specifically, they will determine the pseudospark design characteristics required for medical x-ray production. These characteristics will then be used to suggest a future pseudospark design that should be used in future testing and device development.

Update:

During the first year, we designed, fabricated, and operated a pseudospark plasma source. Photographs of the experimental setup and pseudospark plasma discharge are shown below. Results from the first year of testing showed that 10's of amps of beam current could be extracted from the pseudospark at 40kV charing voltages. In the second year we focused on increasing the voltage and measuring the beam energy. Currently we are testing a new Faraday probe for measuring the energy distribution of the electron beam. The probe is specifically designed to provide a good response at the fast rise times (nsecs) characteristic of the pseudospark. We have also installed a new power supply that allows operation up to 100kV voltages necessary for biomedical x-ray applications.

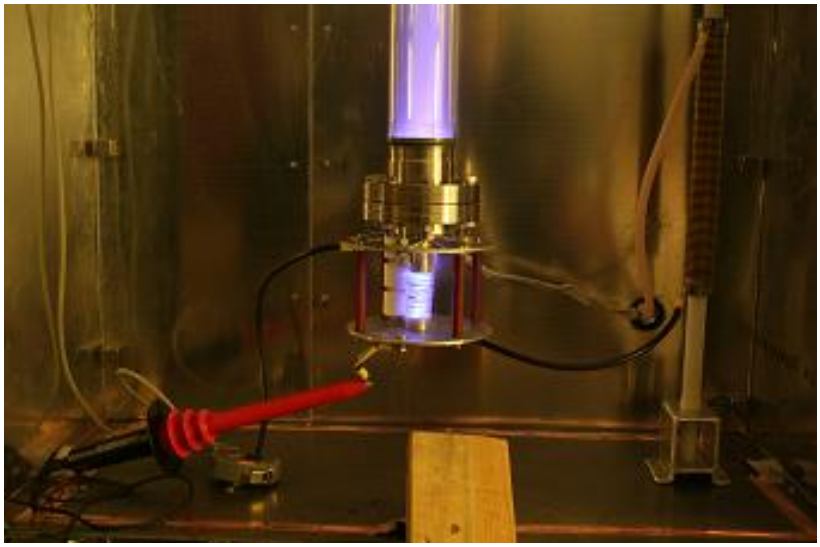


Photo courtesy of Missouri University of Science and Technology.

Project #:	09-1065
Project Title:	Informatics Research Core Facility
Award Amount:	$1,302,217
Center for Excellence:	Statewide
Lead Investigator:	Dr. Mark McIntosh, University of Missouri
Collaborators:	Dr. Chi-Ren Shyu, University of Missouri

Summary:

The objective of this program is to create an **Informatics Resource Core Facility (IRCF)** to enhance Life Sciences research projects across Missouri. The shared resource will serve as the focal point from which research teams can access and gain bioinformatics expertise for the design, data management, and analysis of their research projects and a data warehouse to store their research data sets and resulting analyses. The IRCF will be staffed with trained informatics and biostatistical expertise who will be work synergistically with the MU Informatics Institute, the UM Bioinformatics Consortium and the Office of Research system of Research Core Facilities to insure its technological and training resources are in tune with current developments in advanced statistical analysis of such massive data sets.

To establish this critical and valuable research resource, we proposed a three-year project to accomplish three primary objectives:

1. Recruit trained informatics personnel to provide guidance and expertise in data analysis and interpretation and in biostatistics.
2. Provide computational resources (hardware and software) to link high throughput instrumentation in the Core Facilities to the UMBC data storage and computing resources.
3. Coordinate with MU Informatics Institute to help educate current and future life sciences investigators in the principles of bioinformatics and biostatistical analysis of large data sets.

Once established, the IRCF will be sustained through cost-based charge paid by grants, contracts and other public and private research funds.

Update:

The following specific activities have been directed toward meeting the objectives of the award:

1. Negotiations with Scott Givan were successful and he started as the Associate Director of the IRCF on May 3, 2010.
2. Subsequent to discussions with the IRCF Oversight Committee, the IRCF advertised nationally for two Bioinformatics Analyst positions. Scott Givan assembled an interview panel and the top 5 candidates were interviewed. When necessary, interviews were held remotely, either via telephone or internet chat client (one candidate was hearing impaired). The top two candidates, who happened to be local, were offered the positions and both accepted. Bill Spollen and Christopher Bottoms started on Sept 1, 2010.
3. Scott Givan devised a fee structure for IRCF services. The fee structure is designed to recover the IRCF costs of delivering bioinformatics services, but still be palatable to the clients. The basic structure of the fees is tiered and depends on how soon a client needs the data and the funding for the job (commercial vs. federal/state). Clients are charged lower fees for advanced planning and for state or federal funding; the lowest rates are applied to publicly-funded clients who notify the IRCF more than a month before data analysis is needed. Fees range between $53/hr to $295/hr.
4. Scott Givan is currently working with Leesa Ianke to establish an MU Service Operation account to receive payments from clients.
5. Between September and December 31, 2010, several manuscripts citing IRCF staff have been published or accepted for publication:
    a. Kimbrel J.A., **Givan S.A.**, Halgren A.B., Creason A.L., Mills D.I., Banowetz G.M., Armstrong D.J., Chang J.H. (2010). An improved, high-quality draft genome sequence of the Germination-Arrest Factor-producing *Pseudomonas fluorescens* WH6. BMC Genomics 2010, 11:522.
    b. Shulaev V., et al. (2011). The genome of a woodland strawberry (*Fragaria vesca*). Nature Genetics 2010, doi:10.1038/ng.740. [**Scott A. Givan** is a coauthor]
    c. Chang J., Kimbrel J., **Givan S.**, Temple T., Johnson K. (2010). Genome sequencing and comparative analysis of the carrot bacterial blight pathogen, *Xanthomonas hortorum* pv. *carotae* M081, for insights into pathogenicity and applications in molecular diagnostics. Accepted by Molecular Plant Pathology.
    d. Isom SC, **Spollen WG**, Blake SM, Bauer BK, Springer GK, Prather RS. Transcriptional profiling of day 12 porcine embryonic disc and trophectoderm samples using ultra-deep sequencing technologies. Molecular Reproduction and Development 2010 Sep;77(9):812-9.
    e. **Bottoms CA**, Flint-Garcia S, McMullen MD. IView: Introgression Library Visualization and Query Tool. BMC Bioinformatics 2010, 11(Suppl 6):S28.
6. Scott Givan runs a Next Generation Sequencing user group that meets monthly.
7. IRCF staff presented a poster during the "MO Technology Expo" on October 7, 2010.
8. Chi-Ren Shyu, Gordon Springer and Scott Givan finalized the details of a "Memorandum of Understanding" that will govern the use and management of an IBM x3850 server (purchased with University funds) and any future computational hardware purchased by or dedicated to the IRCF.
9. Scott Givan applied for and was granted time on the Teragrid, a shared national computational infrastructure. Resources in the Teragrid are extensive and largely unavailable elsewhere. Specifically, machines with terabytes of RAM will be used for several large-scale analyses using massive quantities of Illumina-derived DNA sequence.
10. Chi-Ren Shyu and Scott Givan hosted an "Informatics Town Hall" to present the IRCF and receive feedback about the informatics and computational needs of researchers on campus.

The funding has allowed MU to establish an Informatics Core Facility to provide statewide institutions with expertise in database management and analysis relative to life science research. Already, core staff have been contracted to collaborate with investigators at several Missouri institutions and with IBM for this purpose. Several grants are in the development stage that would not have been possible without this informatics expertise. These grants include analysis of genome and proteomic data from agricultural projects, including maize, soybean, cattle, and swine.

Project #:	09-1076
Project Title:	St. Louis Institute for Nanomedicine
Award Amount:	$1,500,000
Center for Excellence:	St. Louis
Lead Investigator:	Dr. Sam Wickline. Washington University
Collaborators:	Dr. Karen L. Wooley and Dr. Dong Qin, Washington University

Summary:
The recent emergence of nanoscience as a key approach to innovation in advanced materials has sparked a similar interest in the application of its principles to the fields of biomedical diagnostics, therapeutics, and basic cell physiology. The overarching goal of the proposed St. Louis Institute for Nanomedicine is to advance the safe and effective use of nanotechnologies to reduce death and suffering from human disease. The global theme that will integrate and focus the planned Institute is *Translation* through development and preclinical evaluation of new nanotechnologies for health care, assessment of safety in production and utilization, facilitation of technology transfer and clinical trials, and education of a new workforce and the public at large. Resources and infrastructure that will be developed to facilitate interdisciplinary implementation of nanomedical devices through collaboration by sponsoring:

1. Research: to expand basic research in nanoscale materials, structures, devices, and pharmaceuticals that may be of benefit for understanding, diagnosing, treating, or preventing human disease;
2. Translation: to guide translation of laboratory breakthroughs into clinical trials by demonstrating safety and efficacy of nanomedical devices and products;
3. Technology Transfer and Commercialization: to foster an entrepreneurial pathway for local commercialization of nanomedical products; and
4. Education and Workforce Development: to train the next generation of scientists and clinicians who will invent and use novel nanomedical products, expand the local talent pool for translation and commercialization, and bring relevant information to the public regarding new developments in nanomedicine.

Specifically, a regional Institute will be created with an inclusive, open network organizational structure: a synergetic environment that should cultivate and enable innovative research directions and translate breakthrough scientific discoveries into practical applications of nanotechnology in the areas of clinical imaging, diagnostics and drug discovery and delivery. They will leverage on their current multi-institutional and multidisciplinary strengths primarily in the development of nanoscale devices to probe and treat cardiovascular disease and cancer and build translational research to generate new commercial opportunities to maximize the regional economic benefits of investments by the state, city, and regional academic institutions and industry partners in medical nanotechnologies.

Update:
The St. Louis Institute of Nanomedicine (SLIN) held a seminar on February 13, 2009 to foster collaboration between the local leading institutions, industry, and individuals. There were approximately 150 attendees. The symposium included a formal program, interactive seminars and opportunities for informal discussions with colleagues. Topics Included: Nano Diagnostics, Regional Infrastructure, and Nano Therapeutics plus breakout sessions on Opportunities for Commercialization, Education/Training Science Center, and Challenges to Translation.

SLIN supported the 2nd Annual Missouri NanoFrontiers Symposium which was co-hosted by Washington University and the University of Missouri-St. Louis on October 27, 2010 at the Danforth Campus.

A key long-term commitment of the Institute is the identification and funding of pilot projects to expand the portfolio of nanomedicine ideas and attract new talent to grow the regional nanomedicine infrastructure. The website for the Institute is currently under construction and can be found at nanomed.wustl.edu/index.html. The St. Louis Institute of Nanomedicine has supported over $600,000 in funding for pilot projects. The progress for these projects is listed below. This funding has supported research as well as undergraduate multidisciplinary curriculum in nanomedicine.

PILOT GRANTS

2011 **Requested** Pilot Grants (Period 1/1/11 thru12/31/11)

PI	Institution	Title of Grant Submission	Direct	Overhead	Total
Qin, Dong	WU	Engineering Silver & Gold Nanoparticles for Biophotonics Applications	$30,000.00	$7,500.00	$37,500.00
Pan, Hua	WU	Development of Nanoparticle Multiplexing Strategy for Rapid Clinic Translation	$30,000.00	$7,500.00	$37,500.00
Hood, Joshua	WU	Inhibiting Sentinel Node Melanoma Niche Progression with Mellittin Modified Exosomes	$30,000.00	$7,500.00	$37,500.00
Chen, Junjie	WU	Anti-angiogenesis Nanomedicine of Proliferative Retinopathy	$30,000.00	$7,500.00	$37,500.00
Singamaneni, Srikanth	WU	Molecular Imprinted Nanostructures for Rapid and Non-Invasive Detection of Kidney Cancer	$30,000.00	$7,500.00	$37,500.00
					$187,500.00

2010 Approved Pilot Grants Period (3-10 thru 3-11)

PI	Institution	Title of Grant Submission	Direct	Overhead	Total
Pan, Dipanjan	WU(2)	Development and characterization of K-edge metal nanocolloids (NanoK) for detection of thrombus with Spectral Computed Tomography (Spectral CT)	$35,000.00	$8,750.00	$43,750.00
Xiong, Yujie	WU(3)	Correlation of Metallic Nanoparticle Toxicity with Physiochemical Properties	$29,000.00	$7,250.00	$36,250.00
George, Thomas	UMSL/STLCC	Undergraduate Multidisciplinary Curriculum in Nanomedicine Phase 2: Real and Virtual Lab Experiments on Nanomedicine	$35,000.00	$8,750.00	$43,750.00
Montano, Adriana	SLU	siRNA based substrate reduction therapy for Morquio A disease	$35,000.00	$8,750.00	$43,750.00
					$167,500.00

2009 Approved Pilot Grants (Periord 8/09 thru 12/10)

PI	Institution	Title of Grant Submission	Direct	Overhead	Total
Gokel, George	UMSL	Pyrogallarene Nanosponges as Ultrasound-switchable Drug Capsules	$60,000.00	$5,000.00	$65,000.00
George, Thomas	UMSL/SLCC	Undergraduate Multidisciplinary Curriculum in Nanomedicine	$60,000.00	$5,000.00	$65,000.00
Tomatsu, Shuji	SLU	Micellar-polymer based enzyme replacement therapy for Morquio A Disease	$20,000.00	$5,000.00	$25,000.00
Shah, Maulik	SLU	Controlled BioErosion of Targeted Zinc Nanodots for Cancer	$20,000.00	$5,000.00	$25,000.00
Harkins, Amy	SLU	Quantum Dots to Investigate Neuronal Communication as an Environmental Sensor	$20,000.00	$5,000.00	$25,000.00
Chen, Daren	WU	On-Chip, Ultra-high-quality Optical Resonator Platform for Single Nanoparticle Detection and Monitoring	$20,656.00	$5,164.00	$25,820.00
Tang, Yinjie	WU	Study on Inhibitory Mechanism of Metallic Nanoparticles to Pathogenic Microorganisms	$14,754.00	$3,688.00	$18,442.00
Xia, Younan	WU	Immuno Gold Nanocages as Molecular Contrast Agents for Early Cancer Detection by Photoacoustic Imaging	$24,590.00	$6,148.00	$30,738.00
					$280,000.00

Project #:	09-1078
Project Title:	Computational simulation of canine biomechanically induced unicompartmental osteoarthritis: a concurrent multiscale approach
Award Amount:	$556,957
Center for Excellence:	Kansas City
Lead Investigator:	Dr. Trent Guess, University of Missouri-Kansas City
Collaborators:	Dr. James Cook, University of Missouri; Dr. Reza Derakhshani, University of Missouri-Kansas City; and Dr. Ganesh Thiagarajan, University of Missouri-Kansas City

Summary:
In 2005, 32% of Missourians (1.38 million people) reported having doctor-diagnosed arthritis (information collected from the Behavior Risk Factor Surveillance System). In addition, 60% of Missourians over age 65 have been told by a doctor or health care professional that they have some form of arthritis, of which osteoarthritis is the most common. Worldwide, it is suggested that approximately 400 million people suffer from osteoarthritis (data from the National Institutes of Health and The Arthritis Foundation).

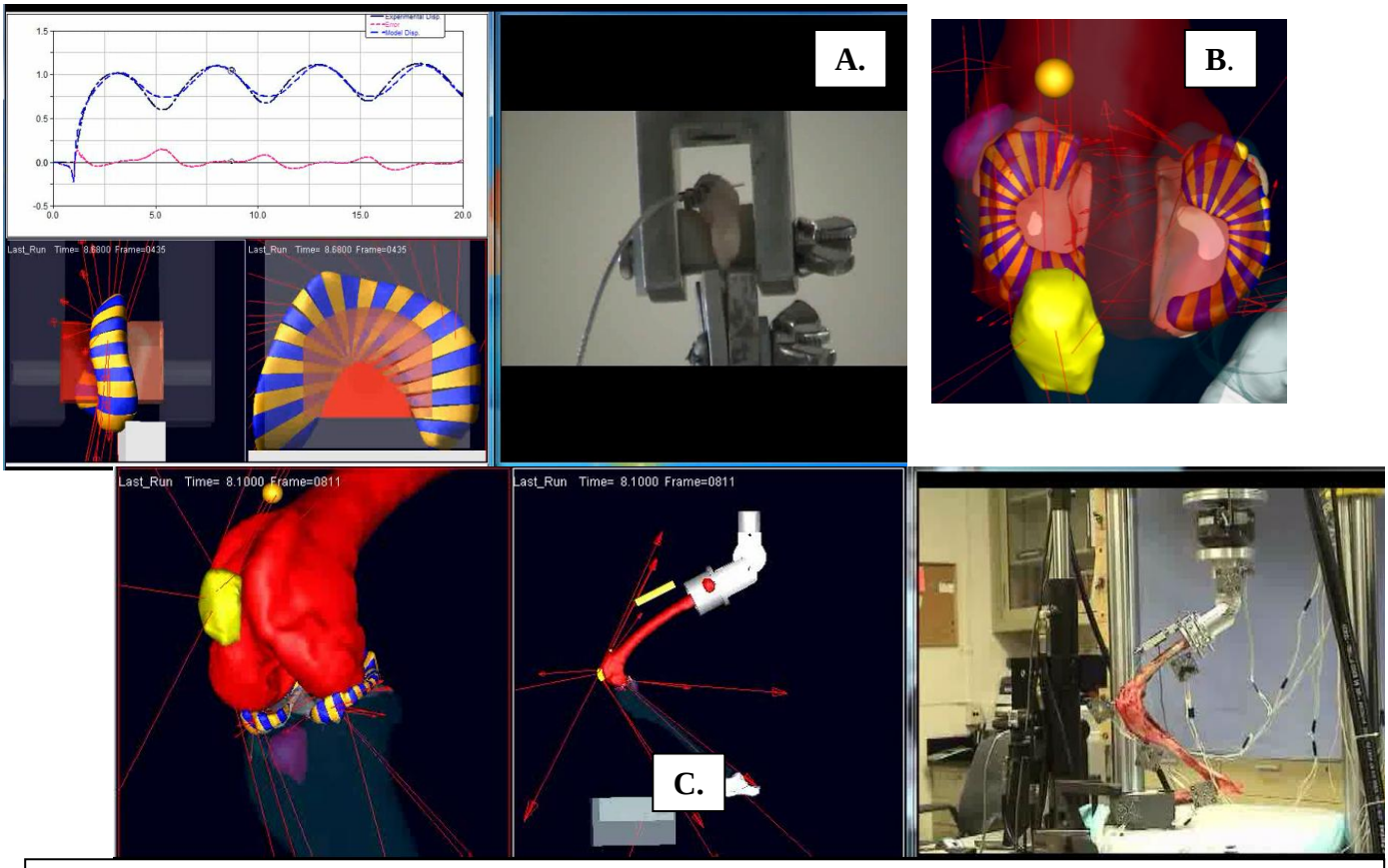
There is no known cure for arthritis and estimates of the total annual cost in the US exceed $90 billion. Osteoarthritis is a debilitating disease that is not completely understood, but evidence links the severity, progression, and treatment of the disease to the mechanical environment in the knee during everyday activities such as walking, running, and stair climbing. The natural response of articular cartilage to insult or injury is an outcome of complex interconnected factors that include anatomy, biology, and muscle forces. The goal of this project is to develop a predictive, computationally efficient, patient level simulation tool of mechanical osteoarthritis indicators. Specifically, a computational model of the canine knee (stifle) that includes surrogate models of cartilage tissue behavior will be combined with musculoskeletal models of movement and validated through in-vivo canine models of osteoarthritis. The computationally efficient cartilage surrogates will predict key tissue indicators of osteoarthritis (shear stress, peak stress, and stress transients) in response to organ level loading and learn from a finite element model solution database.

This work combines the internationally recognized expertise in canine osteoarthritis and tissue engineering of the University of Missouri-Columbia with the musculoskeletal biomechanics expertise and innovative multiscale modeling techniques of the University of Missouri–Kansas City. The project will enhance the reputation and research capabilities of both institutions while developing validated simulation tools of the canine neuromusculoskeletal system that include concurrent tissue level response. This research will enable the development of patient-specific models that predict tissue level mechanical osteoarthritis indicators during movement and simulation tools where tissue level parameters are incorporated in optimization schemes of muscle activation. This project addresses a key area in osteoarthritis research that has largely been neglected, the role of muscles in osteoarthritis pathomechanics including muscle activation patterns and muscle strength.

Update:

This work is the first to develop computational musculoskeletal models of canine gait with anatomical models of the stifle (knee) and hind limb. This research is providing critical information on the biomechanical environment of the stifle with applications to veterinary medicine and translational research for human joint degenerative disease and injury. For example, our research has provided, for the first time, information on changes in mechanical loading of the articular cartilage in a key translational animal model of osteoarthritis with validation of computational modeling using both cadaveric and in vivo assessments. The research is also being used to help address current clinical controversies and problems in veterinary surgery such that pets will benefit from this work as well. The project is a collaboration between the Musculoskeletal Biomechanics Research Laboratory at the University of Missouri–Kansas City and the Comparative Orthopaedics Laboratory at the University of Missouri–Columbia. Project work progress includes:

1) Mechanical testing to determine material properties of the canine menisci and articular cartilage;
2) Magnetic Resonance Imaging and generation of hind limb bone, cartilage, ligament, and menisci geometries;
3) Gait testing at the UMKC Human Motion lab both pre-surgery and post-surgery;
4) Meniscal release procedure to induce unicompartmental osteoarthritis;
5) Development of stifle, hind limb, and musculoskeletal models on two subjects;
6) Experimental testing to validate developed stifle and hind limb models;
7) Development of tissue level finite element models of cartilage indentation testing; and
8) Development of surrogate models of cartilage that learn from finite element solutions, the surrogate models are placed in musculoskeletal models of canine gait for prediction of tissue level stress during movement.



A. Experimental testing and computational model of canine meniscus. **B.** Computational simulation showing contact on the tibial plateau of the canine stifle. **C.** Validation of the computational model of canine hind limb.

Project #:	09-1101
Project Title:	UMKC Center of Excellence in Mineralized Tissues
Award Amount:	$1,050,196
Center for Excellence:	Kansas City
Lead Investigator:	Dr. Lynda Bonewald, University of Missouri-Kansas City

Summary:
Diseases of mineralized tissues such as bone and teeth or of the muscles that control bone movement result in significant health costs in terms of suffering, loss of work and productivity, and even death. There is a tremendous need for new approaches to treating musculoskeletal diseases. Of the 57.9 million Americans injured annually, more than one-half incur injuries to the musculoskeletal system. The most common bone disease is osteoporosis, which leads to fragile bones that break easily. Hip fractures account for 300,000 hospitalizations per year; 20% of those patients die within a year and 20% are relegated to long-term care facilities such as a nursing home. Associated muscle weakness and wasting compound the consequences of immobility. Biomaterials will be required for the estimated 500,000 joint replacements performed annually, and new surgical techniques and rehabilitation strategies will help speed patient recovery.

Scientists are making important discoveries in the lab that could be used to treat patients with diseases of mineralized tissue. Conditions such as obesity and diseases such as cancer can have a devastating impact on the health of the bones, teeth, and muscles and affect patients of all backgrounds and ages. The United States is facing a national epidemic with regards to obesity. Obesity and inactivity in the population are

leading to fragile, weak bones in children and account for more than 300,000 deaths per year. While new discoveries are being made as to how exercise promotes musculoskeletal strength, the population is increasingly sedentary. Injuries, chronic disease, and obesity all result in long-term declines in skeletal and muscle strength. Basic understandings of how bone cells respond to mechanical load and the cross-talk with associated muscles will help to define therapeutic strategies for combating changes in skeletal microarchitecture brought on by inactivity.

Other diseases of mineralized tissue include dental diseases and craniofacial conditions that require procedures ranging from tooth restorations to major reconstruction of facial hard and soft tissues. Children lose more than 50 million school hours and adults close to 200 million work hours each year due to dental visits because of deteriorating oral conditions and dental disease. Improved biomaterials technology is needed to develop new approaches for treatment.

The formal establishment and support of a UMKC Center of Excellence in the Study of Mineralized Tissues would accelerate new discoveries and convert these discoveries into therapies and treatment serving the health and welfare of the residents of the state of Missouri. This Center of Excellence will be structured to leverage significant existing resources to generate a mechanism to increase productivity and funding to a much greater extent over that which would be expected from the individual components and constituents. Funding of the present application would provide seed money for projects with high potential for technology transfer and for interdependent, collaborative projects within the Center of Excellence that will lead to federal grants and support from industry. In summary, this Center will build upon the success of its members to create an environment supportive of creative discovery that will attract prominent researchers and facilitate transfer of discoveries to industry and to the patient.

Update:

The Center monthly seminar meetings are the third Wednesday of each month: The UMKC Center of Excellence in Mineralized Tissues Series. The Center Leadership team has established a meeting every third month to discuss the focus and direction of the Center. Two of the Center projects (8 and 11) have received outside funds based on data generated with Missouri Life Sciences Resource Board support.

The goal and purpose of the UMKC Center of Excellence in the Study of Dental and Musculoskeletal Tissues is to accelerate new discoveries and convert these discoveries into therapies and treatment serving the health and welfare of the residents of the state of Missouri. This Center of Excellence is structured to leverage significant existing resources to generate a mechanism to increase productivity and funding to a much greater extent over that which would be expected from the individual components and constituents. Funding is sought to provide seed money for projects with high potential for technology transfer and for interdependent, collaborative projects that will lead to federal grants and support from industry. The Center strives to create an environment supportive of creative discovery that will attract prominent researchers and facilitate transfer of discoveries to industry and to the patient.

Members of this center, approximately 30, are from the Schools of Dentistry, Medicine, Nursing, Chemistry, and Computing and Engineering. Their total funding is approximately $30 million. The Center has yielded significant returns. Fifteen pilot projects have received funding after being approved by the internal review committees.

Many of the projects were able to attract additional federal and private grant funding totaling over $5 million. Several papers have been published with many still to be submitted. This is a significant return on the investment by the MLSRB. For more information about specific projects visit the Center website at http://cemt.umkc.edu.

Project #:	09-1105
Project Title:	Acquisition of Metabolomics Platform for Metabolic Engineering
Award Amount:	$894,993
Center for Excellence:	St. Louis
Lead Investigator:	Dr. Leslie Hicks, Donald Danforth Plant Science Center

Summary:
The ability to detect, identify, and characterize biomolecules remains an essential component in the elucidation of complex cellular processes. Progress in the field of analytical instrumentation development continually advances current capabilities and the ease by which these efforts can be carried out. The purpose of this proposal was to seek funding for instrumentation to establish a robust, high-throughput metabolomics platform at the Danforth Center. The platform needed to suit the various needs of multiple plant biologists, but overall superior sensitivity, dynamic range, and mass accuracy were essential for successful project completion and thus in securing future research funding.

The Donald Danforth Plant Science Center is an independent, non-profit research institution conducting interdisciplinary plant science research in genetics, biochemistry, cell biology, physiology, and structural biology. Established in 1998, the Danforth Center is a 170,000 sq. ft facility constructed on a 40-acre site located in St. Louis, Missouri. The Danforth Center includes state-of-the-art research laboratories, green houses, growth chambers, and three core scientific facilities that service the needs of scientists in the areas of microscopy, tissue culture, and proteomics/mass spectrometry. It has strong educational and research partnerships and has established active international research and training programs. The Danforth Center provides infrastructure that enables training of scientists, postdoctoral fellows, and graduate/ undergraduate students, accomplishing one of the guiding tenets of the Danforth Center charter: to provide a world-class institute where scientists from around the world can learn, train, and conduct research to answer some of the most important questions in plant biology.

Update:
With the award of this grant for the purchase of instrumentation, the PI leveraged the funds to purchase additional and more advanced instrumentation with the grant funds than originally proposed. The grant originally requested the purchase of one TOF and one QTOF instrument, but we were able to purchase two QTOF instruments, one GCMS, and auxiliary separations equipment to further enhance our metabolomics capabilities and facilitate our platform development. All instrumentation was ordered, installed and operational within 9 months, and we are now continuing with method(s) development, optimization, and applications (including sample preparation, LC separations, instrument and data analysis methods) on an ongoing basis. These ongoing development efforts are funded via the DOE-EFRC Center for Advanced Biofuels Systems (CABS) program (Hicks, coPI) for rational engineering efforts in biofuels research for algae and camelina.

Statement of Results:
The award of this funding has and will continue to positively impact research in the areas of natural product biosynthesis, plant/microbe interactions, plant lipidomics, metabolic flux and metabolic engineering for enhanced biofuel systems. Additionally, it facilitates plant biology researchers at the Danforth Center and collaborators to further expand their research scope into the interface of biology and chemistry and promotes the creation of cross-disciplinary specific aims and collaborations that will facilitate a macroview analysis of specific projects while deriving systems-wide information.

Project #: 09-1106
Project Title: Drought Simulators Critical to Translational Research in Plant Science
Award Amount: $1,558,125
Center for Excellence: Statewide
Lead Investigator: Dr. Felix Fritschi, University of Missouri
Collaborators: Dr. Robert Kallenbach, University of Missouri; Dr. Grover Shannon, Delta Research Center; Dr. Stephen Anderson, University of Missouri; Dr. Deborah Finke, University of Missouri; Dr. Robert Kremer, University of Missouri; Dr. Randall Miles, University of Missouri; Dr. Henry Nguyen, University of Missouri; Dr. Melvin Oliver, University of Missouri; Dr. Craig Roberts, University of Missouri; Dr. David Sleper, University of Missouri; Dr. William Stevens, University of Missouri; Dr. Kelly Tindal, University of Missouri; Dr. William Lebold, University of Missouri; Dr. Allan Wrather, University of Missouri; and Dr. Xi Xiong, University of Missouri

Summary:
Water is a finite resource that is in great demand for a wide variety of reasons, including domestic, industrial, leisure, and agricultural uses. In light of population increases and greater demands for non-agricultural uses, more and more emphasis will have to be placed on efficient use of water resources available for plant production. Presently, facilities to reliably examine plant responses to drought under field conditions do not exist in Missouri. However, to translate research findings developed in controlled environment facilities to field conditions, the ability to manage the timing, duration, and intensity of water deficit stress under field conditions is essential. The drought simulators currently under development are critical in bridging the gap between laboratory based experiments and conditions encountered in the field. Once completed, Missouri researchers will have access to a unique network of drought simulators that will allow them to address a broad range of topics, including 1) identify new germplasm with increased drought tolerance, 2) identify underlying genetic mechanisms that control drought tolerance, 3) uncover and evaluate physiological mechanisms conferring greater drought tolerance, 4) develop and evaluate cultural practices to increase crop productivity and reduce environmental impact, and 5) study the influence of water deficit stress on soil-plant-insect and soil-plant-pathogen interactions.

Update: The objective of this project is to develop automated drought simulators that will enable Missouri scientists to take new discoveries in basic plant drought research and develop products and practices that will reduce grower losses from lack of rain, study ways to increase water use efficiency, and protect environmental resources. In 2010, the project moved from finalization of the design to construction and completion of two modules at the Bradford Research and Extension Center (BREC) in Columbia, Missouri. The two modules (see picture) at BREC are now in the testing phase and will be fully functional for the 2011 growing season. The field in and around the modules has been fall-tilled and planning of the first experiments to be conducted in 2011 is underway. Planning for the construction of the third module is underway. A suitable field site at the Horticulture and Agroforestry Research Center in New Franklin, Missouri has been identified and surveyed. Design and construction of the third module will benefit from the experience gained during the construction and testing of the two modules at BREC.

Drought Simulators at the Bradford Research and Extension Center in Columbia, MO. (October 2010). The two modules measure 50 x 100 ft and are mounted on tracks that are 350 ft long. The modules are deployed automatically when precipitation is sensed and move to cover one of the research areas within the tracks as defined by the research needs.
Photo courtesy of the University of Missouri.

The project is proceeding as planned and we are looking forward to starting the construction on the third module and are excited about being able to initiate research projects at the BREC drought simulators.

Project #:	09-1117
Project Title:	Advanced Cardiovascular Stent incorporated with Nitric Oxide Delivery System
Award Amount:	$540,000
Center for Excellence:	Kansas City
Lead Investigator:	Dr. Chi Lee, University of Missouri-Kansas City
Collaborators:	Dr. Hai-Lung, Missouri S&T; Dr. Richard Hopkins, Children's Mercy Hospital; and Dr. Yungyun Lee, University of Missouri-Kansas City

Summary:
Cardiovascular stents are metal scaffolds placed in a narrowed atherosclerotic artery to keep the vessel open by providing structural support and prevent it from re-occluding, a condition called restenosis in which endothelial cell growth proliferates around the device as part of the body's natural wound-healing response and impedes blood flow. Most bare metal stents are reported to cause restenosis after a few months of surgery and thus newly developed stents are covered with inhibitory agents to prevent restenosis. Even though the drug-releasing coatings for stents are an exciting protective device that can provide an enormous clinical benefit, the drug coating may peel and delaminate from the metallic surface of the stent due to poor adhesion between the coating and the stent surface. The coating delamination can potentially lead to embolism, acute thrombosis, inflammation and non-uniform delivery of drugs.

In this proposal, they will attempt to reduce restenosis and improve the biocompatibility of a metal scaffold (MS) with two nanotechnology approaches: i) pattern modification by the nano/micromachining technique with a femtosecond laser (FSL) and ii) the development of microparticles (MP) containing Nitric Oxide (NO) using the double emulsion method. The key to this proposed study is the partnership of interdisciplinary scientists in pharmaceutics, engineering, cardiology, advanced proteomics, pharmacological and computer sciences, which seems to be an ideal approach for biomedical research. This proposal is highly innovative in that they will develop the MS with different patterns of the groove in its outer surface which can firmly retain NO loaded MP and exert a controlled release rate of NO according to the loading conditions.

As heart-related diseases remain the most prevalent cause of death in the world, a continuous and controlled supply of NO through MS built with nanotechnology will greatly relieve the long-term risk of cardiovascular diseases. It was also reported that heart disease was the leading cause of death accounting for 16,708 deaths (about 30%) of the Missouri state's deaths in 2002 (National Vital Statistics Report 2004; 53(5)). Therefore, MS built with nanotechnology shall benefit thousands of patients under cardiovascular complications in the state of Missouri by reducing the burden of heart disease and stroke, and promoting activities that can be implemented in health care, communities and schools.

Update:
This project has been successfully performed as proposed in the specific aims. We are sure that the outcome of this project as well as continuous work shall benefit thousands of patients under cardiovascular complications in the state of Missouri by reducing the burden of heart disease and stroke, and promoting activities that can be implemented in health care, communities and schools.

The major hypothesis of this proposal is that a metal scaffold (MS) built with nanotechnologies (i.e., i) loaded with microparticles (MP) containing nitric oxide (NO) prodrugs and ii) competently positioned at the grooves (i.e., produced by nano/micromachining techniques with a femtosecond laser (FSL)) will reduce restenosis and improve biocompatibility. To test this hypothesis, several objectives were established and tested.

The advanced nanotechnology approaches for the Cardiovasular Stent have achieved the following aspects:

i) Improved the blood flow rate;
ii) Increased the cGMP level in cardiovascular cells;
iii) Affected the expression of PKC; and
iv) Reduced restenosis/thrombosis, and improved biocompatibility at the local site.

Future research will be conducted as a result of the research and include: 1. An assessment of advanced stents containing hybrid nitric oxide donors which reduce restenosis/thrombosis aggregation; 2. An assessment of advanced stents made of endogenous substances which maintain physical strength as well as biocompatibility; and 3. An assessment of morphological aspects of cardiovascular vessels upon exposure to advanced stents.

Project #:	09-1128
Project Title:	Targeting Plasminogen Activator Inhibitor-1 to Inhibit Restenosis
Award Amount:	$815,625
Center for Excellence:	Statewide
Lead Investigator:	Dr. William Fay, University of Missouri
Collaborators: Dr. Douglas Bowles, University of Missouri; Dr. Dmitri Baklanov, University of Missouri; Dr. Daniel Lawrence, University of Michigan; and Dr. Brian Wamhoff, University of Virginia	

Summary:
Missouri is among the states with the highest incidences of cardiovascular disease, ranking 8th in 2005. Many patients with coronary artery disease (CAD) undergo percutaneous coronary intervention (PCI), a procedure in which a cardiac catheterization is performed and a stent is placed in a coronary artery at the site of a stenosis (blockage) to improve blood flow. Most patients receive stents that are coated with drugs designed to prevent restenosis--i.e. the re-growth of the blockage within several months after the initial procedure. Drug-eluting stents inhibit growth of cells within the wall of the artery that cause restenosis. However, within the past few years it has been discovered that drug-eluting stents, in the process of inhibiting restenosis, also inhibit the normal repair of the inner lining of the artery (i.e. the endothelium), thereby predisposing the artery to thrombosis, or abnormal blood clotting, which can lead to heart attack and death. The overall objective of this proposal is to develop new strategies to prevent restenosis without increasing the risk of thrombosis.

The project will study novel compounds that inhibit the function of plasminogen activator inhibitor-1 (PAI-1), a key blood clotting protein. These studies will involve the use of pigs, which are widely regarded as the best animal model to test and develop potential treatment strategies for heart disease in humans. The proposed program is multi-disciplinary in nature, involving collaborations between physicians and basic scientists of the University of Missouri-Columbia School of Medicine and College of Veterinary Medicine, as well as investigators at the University of Michigan and University of Virginia. They anticipate that the data generated from the proposed studies will lead to submission of a Small Business Innovation Research (SBIR) application to the National Institutes of Health focusing on development of new compounds to inhibit restenosis in humans without promoting thrombosis. The University of Missouri-Columbia is the only university in the state, and one of the few in the nation, with a medical school and veterinary school located on the same campus. The collaboration between medical- and veterinary based investigators is a major strength of the proposal. This work has great potential for developing pig models of human diseases that will lead to new treatment strategies for patients. They anticipate that their studies will have substantial commercial potential, since new approaches are needed to prevent restenosis without promoting thrombosis. Given the high prevalence of CAD in Missouri, the proposed studies are strategically aligned with the state's health care, economic development, and research opportunity objectives.

Update: The Specific Aims of our project are:

1. Study the effects of compounds that inhibit plasminogen activator inhibitor-1 (PAI-1) on intimal hyperplasia in pigs.
2. Develop and characterize intravascular stents that elute PAI-1-targeting compounds
3. Identify mechanisms by which PAI-1 and pharmacologic targeting of PAI-1 regulate smooth muscle cell migration through 3-dimensional collagen matrices

Aim 1. We have performed balloon angioplasty in approximately 30 animals. We have found that PAI-1-R, a protein, appears to block narrowing of arteries after balloon angioplasty. We have just initiated a round of experiments involving 10 pigs that received either a drug-eluting stent or a control (non-coated) stent. The drug coated on stents was PAI-039, an inhibitor of PAI-1. We will obtain the results of these experiments in January 2011. We are excited about these studies, as they are the first ever performed to test the efficacy of a new form of treatment (local delivery of an inhibitor of PAI-1) on narrowing of arteries after implantation of a stent.

Aim 2. We purchased a stent coater with funds from the Missouri Life Sciences award. We have coated stents with PAI-039. We have collaborated with Brian Wamhoff, Ph.D., University of Virginia, and Nathan Leigh, Ph.D., University of Missouri, to study the release of PAI-039 from coated stents. Our studies show that relatively large amounts of PAI-039 can be coated on stents and be released from them in active form. We are currently performing experiments to study the effect of PAI-039 released from stents on the migration of smooth muscle cells (these are cells present in the walls of artery that migrate in vivo to cause re-narrowing of blood vessels after angioplasty). Our preliminary data suggest that PAI-039 released from stents inhibits smooth muscle cell migration.

Aim 3. We have completed studies examining the effect of PAI-1-R, a protein that inhibits PAI-1 function, on smooth muscle cell migration. This work was published in 2010, as indicated earlier in this report. The trainee who performed this work, Nadish Garg, MD, was selected as a finalist for the Young Investigator Award of the American College of Cardiology in 2010, based on his submission of an abstract and manuscript summarizing the results of experiments funded by the grant.

Project #:	09-1148
Project Title:	Optimization of Camelina as a nonfood production platform of value-added biotechnology products.
Award Amount:	$593,564
Center for Excellence:	St. Louis
Lead Investigator:	Dr. Eliot Herman, Donald Danforth Plant Science Center
Collaborators:	Dr. Roger Beachy, Donald Danforth Plant Science Center; Dr. Monica Schmidt, Donald Danforth Plant Science Center; and Dr. Gene Stevens, Delta Research Center

Summary:

This project proposed to develop *Camelina sativa* as a nonfood industrial crop for Missouri farmers and industrial users. Ideally, a commercially successful industrial crop is one that produces multiple products, each with its own value stream. For example, a plant that produces biodiesel, industrial enzymes, and polymers for plastics will have greater value than one that only produces a single product. The new crop must be adapted to growth and high yields suitable for Missouri farmers. Finally, the crop should be developed to reduce unwanted spread and growth of proprietary materials. This is a collaborative project that brings together basic sciences, biotechnology, and plant breeding. Furthermore, the outcome of the

project is of significant interest to Metabolix Inc., as part of their ongoing research. If successful, this project will also lead to the growth of life sciences companies in the State.

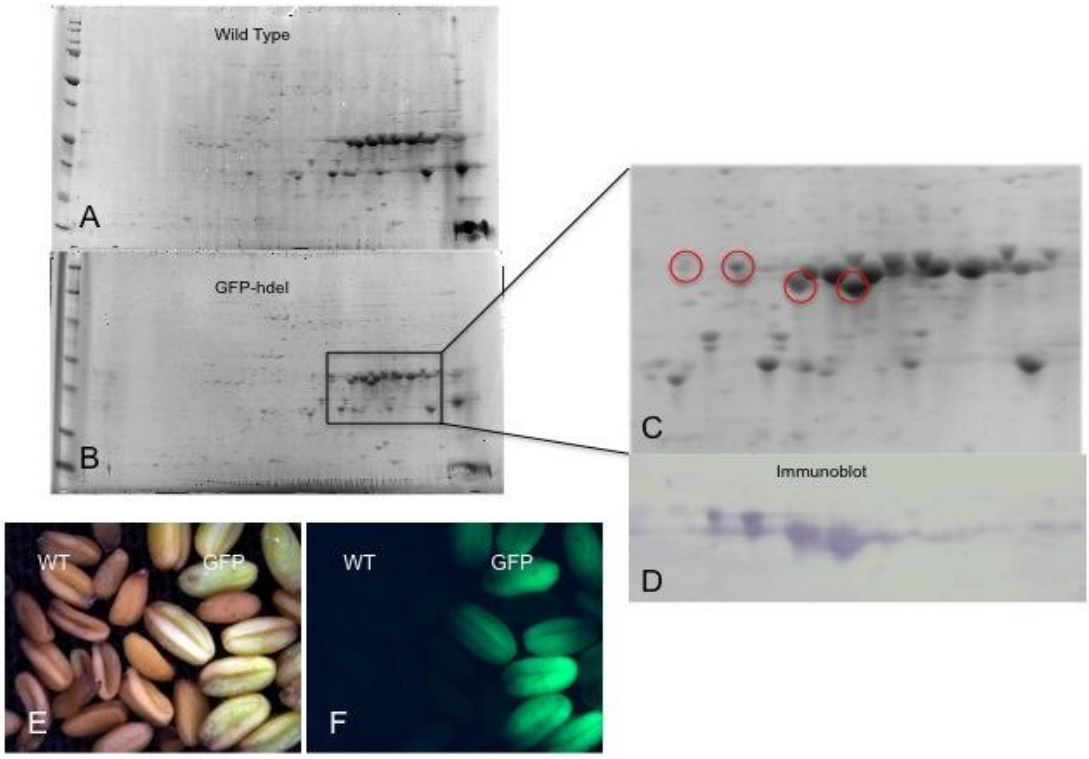
Update:

Dr. Stevens and his group have been testing various accessions of *Camelina sativa* for field performance at various locations in Missouri. These are detailed in the following paragraph. While *Camelina* is easy to grow and transform in contained glass houses, in the field we have had repeated cropping problems due to endogenous disease(s). Over the long term successful production of *Camelina* in Missouri may require selection of resistant lines to the Aster yellows disease. Even with the disease difficulties Dr. Stevens has obtained viable yields of nearly 1000 lb per acre.

At Portageville, Columbia, and Novelty, Missouri, *Camelina sativa* field experiments were infested with an unidentified disease. In 2009, the disease caused leaves of different accession of *Camelina* to turn yellow and orange at flowering, often followed by premature defoliation during pod fill stage. In 2010, the disease symptoms were observed shortly after emergence at Portageville. The problem caused low yields or complete stand losses in tests of variety/germplasm, seeding rate, and nitrogen fertilization. A fungal disease was suspected even though *Camelina* roots, stems, and leaves did not show signs of damping off, rust, or powdery mildew. When the disease was first observed at flowering in 2009, two foliar sprays of azoxystobin (Quadris®), a broad spectrum fungicide, were applied. In 2010, mefenoxam (Ridomil Gold®) was broadcast on the soil surface pre-emergence, followed by azoxystobin foliar applied at flowering. Neither chemical provided control of the disease in the field. The disease was not observed in greenhouse plantings at the Delta Center greenhouse. After further examination and reading reports from other workers who are studying Camelina, including from Canada and Chile, we currently favor the diagnosis of the disease of Aster yellows caused by phytoplasma, a wall-less bacterium. The disease-causing agent is vectored by leafhoppers that feed on phloem of these plants. Missouri farmers considering growing *Camelina* should use an insecticide seed treatment such as fipronil with routine scouting for sucking insects.

Although plot to plot variability was high, we were able to grow CO46, Calena, Ligena varieties and USDA plant accessions (PI650140, PI650144, PI650161, PI650163). PI650144 (Boha), a variety from Denmark, was the best accession tested. The best planting and fertilization treatments were 11 lb seed planted per acre with 90 lb N per acre. Fields were usually too soft and wet for drill planting until late March or early April. Frost seeding in February was tested at Portageville and Novely, but drill seeding provided the most uniform plant stands.

A paper on the prospects of developing an industrial protein production crop, *Camelina*, was published in GM Crops, citation: Herman EM and Schmidt MA (2010) Industrial Protein Production Crops - New Needs and New Opportunities. GM Crops, in press (inaugural cover article) 1:2-7. A figure from that article is shown below.



Photos courtesy of the Donald Danforth Plant Science Center.

FY2009 Funding Summary – Commercialization

Grant Number	Project Title	Principal Investigator	PI Institution	Grant Category	Funds Awarded
09-1011	Molybdenum-99 / Technetium-99m Processing Facility At MURR	Dr. Ralph Butler	University of Missouri/Reactor	Equipment	$1,097,761
09-1024	Translational Development Center	Marcia Mellitz	Center for Emerging Technology/SLU	Equipment	$520,000
09-1034	Photoacoustic detection of circulating melanoma cells in blood	Dr. John Viator	University of Missouri	Project Proposal	$407,789
09-1177	iPrep: Ophthalmic Povidone-Iodine Antiseptic Formulation	Dr. Wendell Scott	St. Johns Medical Institute	Project	$574,450
				TOTAL	**$2,600,000**

FY2009 Commercialization Project Summaries

Project #:	09-1011
Project Title:	Molybdenum-99 / Technetium-99m Processing Facility At MURR
Award Amount:	$1,097,761
Center for Excellence:	Statewide
Lead Investigator:	Dr. Ralph Butler, University of Missouri
Collaborators:	Dr. John Robertson, University of Missouri

Summary:

The University of Missouri Research Reactor (MURR) Center has been working towards becoming a large scale supplier of molybdenum-99 (^{99}Mo) through the fission of low enriched uranium (LEU) targets in the reflector region of the reactor. Feasibility studies indicate that MURR could supply one-half of the US nuclear medicine community's need for ^{99}Mo and its decay daughter product technetium-99m (^{99m}Tc). The intended purpose of the grant was to assist MURR in the development of the conceptual design and cost estimate for the facilities necessary for the handling of irradiated LEU targets and the ^{99}Mo extraction process.

Update:

The MLSRB grant supported the development of an enhanced conceptual design and technical project plan for both a "two-train" and "four-train" facility. (See Figures One and Two) Two designs were developed to enhance marketability to potential investors so as to provide them with an option. The "two-train" facility would have the capacity to produce up to an estimated 2,200 six-day curies of ^{99}Mo per week. The "four-train" facility would have the capacity to produce up to 3,000 six-day curies per week of ^{99}Mo. The grant also supported the development of the project schedules and a work breakdown structure for the project. In general the grant supported several technical meetings and numerous conference calls related to the conceptual design and licensing work.

In addition to the conceptual "two-train – four-train" design work, the grant supported the development of a draft Licensing Plan for the required U.S. Nuclear Regulatory Commission (NRC) license. In May 2009 we presented our licensing approach to the US NRC. We also submitted a Letter of Intent for the project to the US NRC for their records and planning purposes.

The grant supported the development of a Decommissioning cost estimate for both the "two-train" and "four-train" facilities. The US NRC requires a funding plan for decommissioning prior to licensing, thus it was necessary to develop the detailed Decommissioning cost estimate for both conceptual designs. The Decommissioning cost estimate was used in developing the business plan financial model.

The MLSRB grant supported the design development work of the LEU foil targets including target performance yields, configurations, assembly and disassembly methods and failure modes; conceptual design of the irradiation rigs for the targets in the reactor reflector region; the chemical process for dissolving the LEU targets and extracting the ^{99}Mo, waste stream process management (Figure Three) and initiation of the necessary safety analysis as well as conceptual design of the dissolver unit. Below are the conceptual renderings of the ^{99}Mo facility.

Figure One: 3-D Rendering

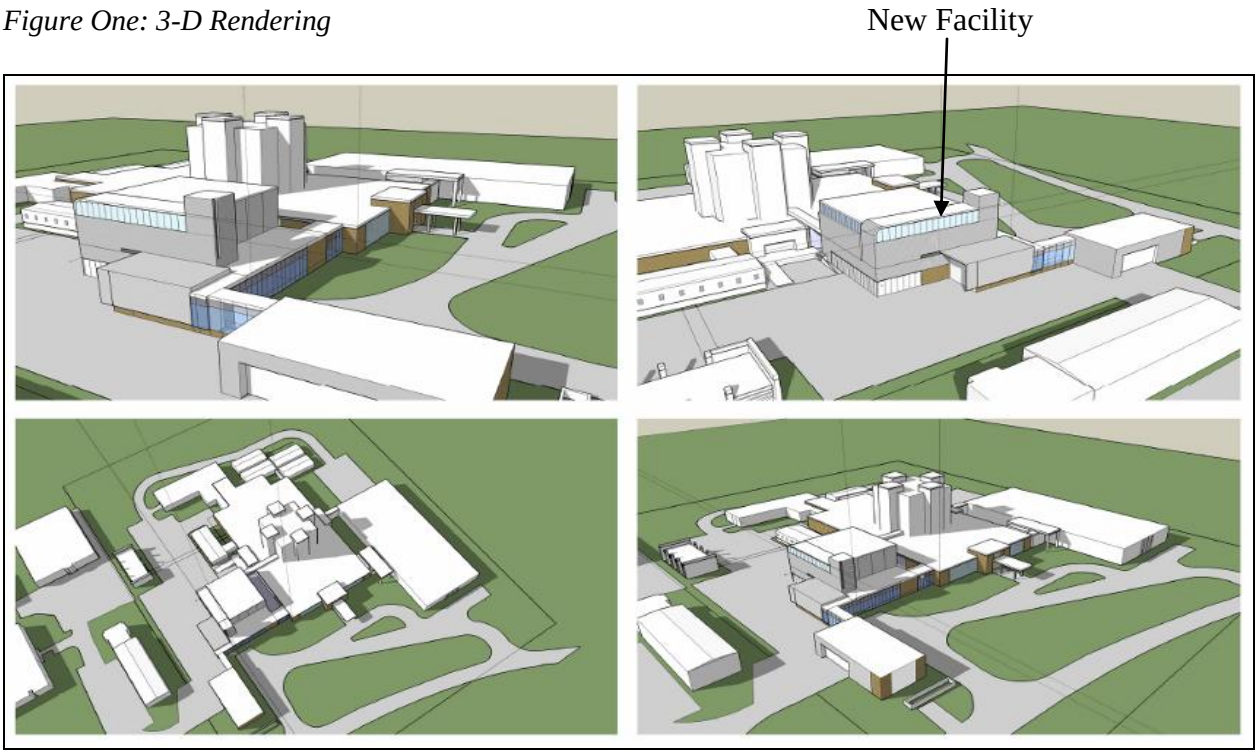


Figure Two: Conceptual Design – 3-D

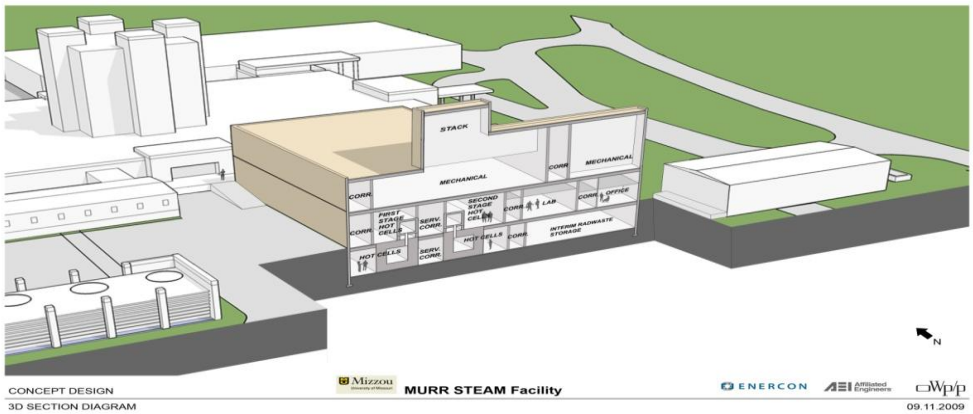
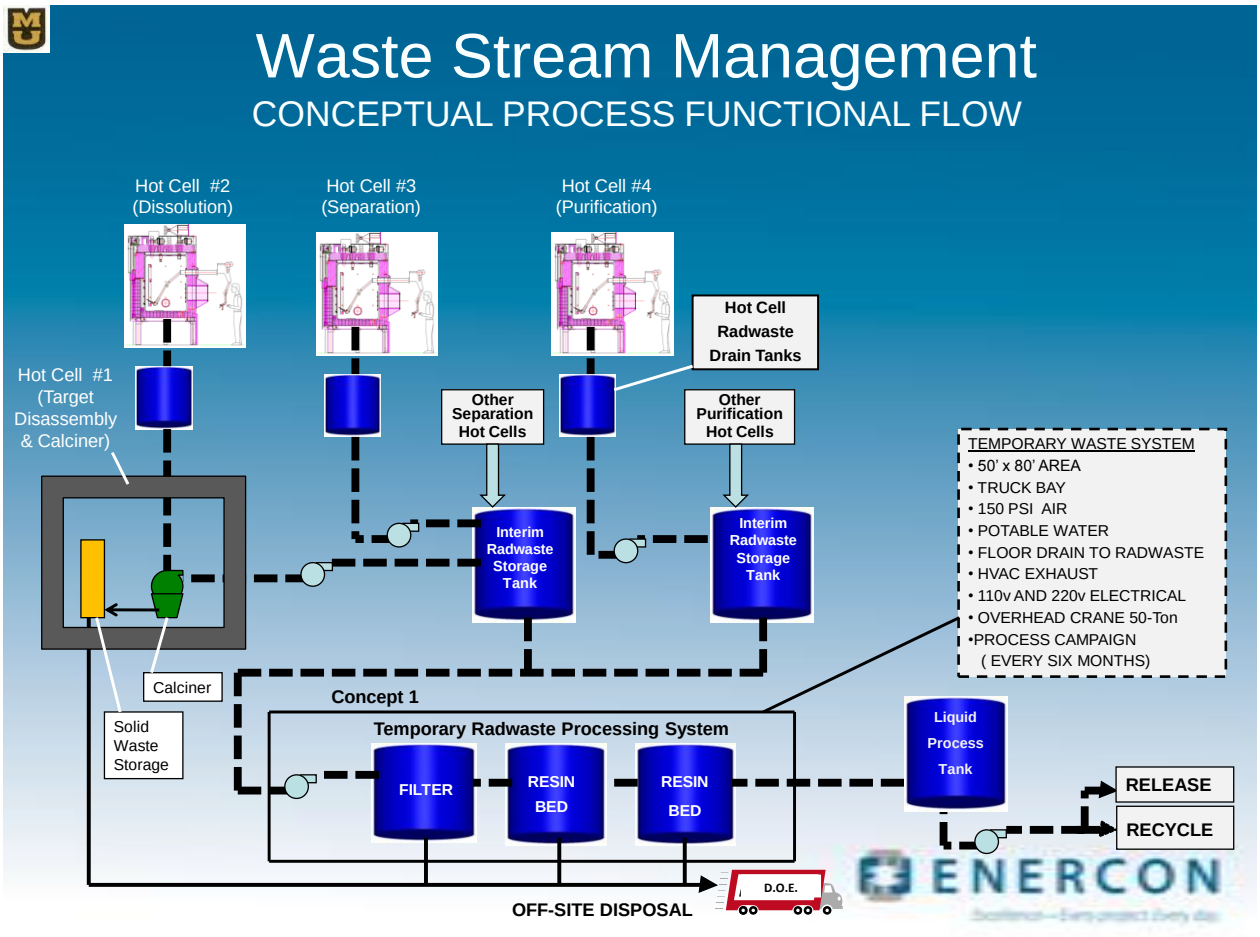


Figure Three: Conceptual Design - Waste Stream Process Management



Statement of Results:

The University of Missouri-Columbia provided matching funds of $250,000 in support of this project. Without the MLSRB grant the matching funds from the University's PRIME Fund would not have been possible. The PRIME funds were partly used to develop a detailed market analysis. The grant supported documents were used to develop a detailed business plan and financial model. The business plan and financial models are being used to solicit both public and private sources for the additional funding necessary for the detailed design, licensing, and construction of the facilities. As an example, the Department of Energy National Nuclear Security administration recently awarded the MU College of Engineering a $473,000 grant to continue the design development effort for the LEU foil targets.

The deliverables produced from this grant have enabled the University to enter into discussions with several potential investors and/or partners for the purpose of obtaining the significant funding required for the commercialization of this project.

Project #:	09-1024
Project Title:	Translational Research Space at CORTEX
Award Amount:	$520,000
Center for Excellence:	St. Louis
Lead Investigator:	Dennis Lower, CORTEX (Transferred from the Center for Emerging Technologies)

Summary:
In 2009 the Center for Emerging Technologies (CET) was the recipient of a three-year MLSRB commercialization grant for its Translational Development Center. The grant funds were dedicated to fitting-out and equipping web lab research and associated office space in Building III which was being developed adjacent to CET's existing facility. Although CET expects to develop Building III in the future, the recent economic downturn has prevented CET from moving ahead with its immediate construction as planned. CET identified the critical need for translational research space in the region due to the surge of laid-off Pfizer employees wanting to start new bioscience companies.

Update:
In April of 2010, CET requested the transfer of the MLSRB grant to the Center of Research, Technology and Entrepreneurial Exchange (CORTEX) in order to build out vacant space as translational research space. The funds will be used to fit out and equip 5400 sq ft of translational research laboratory and associated office space. The MLSRB approved this request in July 2010. The Biogenerator Accelerator Labs at CORTEX provides researchers and entrepreneurs access of critical lab space and equipment to translate their ideas from bench to market at a low cost. Wet laboratory space includes specialized spaces for medicinal chemistry and cell biology, along with general purpose lab space. Since opening in September 2010, five early stage life science companies have initiated operations in the new labs.

First tenants include Confluence Life Sciences, a drug discovery company founded by a team of former Pfizer scientists, SARmont, a medicinal chemistry contract research organization, and Six Convert, a new startup commercializing a novel technology to enable wastewater-to-energy projects.

BioGenerator ribbon-cutting ceremony. ***Photo courtesy of CORTEX.***

Project #:	09-1034
Project Title:	Photoacoustic detection of circulating melanoma cells in blood
Award Amount:	$407,789
Center for Excellence:	Statewide
Lead Investigator:	Dr. John Viator, University of Missouri
Collaborators:	Dr. Paul Dale, Dr. Scott Holan, and Dr. Luis Polo-Parada, University of Missouri

Summary:
Melanoma is the deadliest form of skin cancer and has the fastest growth rate of all cancer types. In the U.S., the lifetime risk is about 1 in 55, while other parts of the world have even greater risks. Early surgical resection of melanoma is the best avenue of therapy. However, for those cases where the lesion progresses and spreads, monitoring of metastatic disease is crucial for positive clinical outcomes.

Detection of circulating melanoma cells (CMC's) in human blood and lymph systems has the potential to find early metastasis and monitor therapy. They have detected CMC's by generating photoacoustic waves, or laser induced ultrasound, in melanoma cells suspended in saline and in single CMC's in blood samples from advanced melanoma patients. They will improve their current photoacoustic detection device by incorporating an optical reactance acoustic sensor in their apparatus and by implementing a two color laser method for increasing the ability to specifically find melanoma. These improvements will increase accuracy for single melanoma cell detection of lightly pigmented cells. They will also alleviate alignment problems, allowing robust detection of CMC's in a clinical device. The ability to detect single CMC's makes this device a viable tool for monitoring metastatic melanoma cells in blood.

Melanin, a strong optical absorber within melanoma cells, absorbs low energy, rapid laser pulses thereby generating photoacoustic waves. The procedure for CMC detection would be performed by separating the white blood cells from a melanoma patient's blood sample. CMC's, if present, reside among the white blood cells. These cells are introduced into the photoacoustic detection system and are scanned for melanoma cells. In a healthy sample in which there are no melanoma cells, no acoustic pulses will be generated. In a sample containing metastatic melanoma, high frequency acoustic pulses would be generated and detected by the system.

This detection system manifests as two marketable products. The first product is the laser detection system described above that will be located at centralized testing centers, costing about $50,000-100,000. The second product is a CMC test kit costing approximately $50. The test kits would be used for regular monitoring of the 600,000 melanoma patients in the U.S. and by millions in the international markets. In this study, they will test up approximately 40 Stage IV melanoma patients during years 2 and 3. The actual number will depend on analysis of a pilot study currently underway. They will correlate disease state and survival using Kaplan-Meier plots to the number of CMC's detected in their blood samples. Using the results of this study, they will pursue FDA pre-market notification (510(k)) for the melanoma detector as a method to monitor disease state in advanced patients. This 510(k) strategy is conservative, with a high likelihood of being obtained. With subsequent funding from the SBIR and investor capital, they will conduct more efficacy studies to obtain 510(k) for detection of early metastasis.

Two objectives were stated for this proposal: 1) Improve the photoacoustic detection system for robust detection of single CMC's, and 2) Perform a prospective clinical study correlating disease state and survival to numbers of CMC's detected in blood samples of Stage IV melanoma patients. The first year of this effort has been spent on the first objective and all of the facets of this objective have been performed successfully. The subsets of this objective and details of accomplishment are described below:

(1) The apparatus for optical detection of photoacoustic waves has been built and tested on melanoma cells from cell culture and from limited numbers of stage IV melanoma patients. The

results of testing this device have been submitted to "Lasers in Surgery in Medicine" and "International Journal of Thermophysics". We have detected single cell events using this device in static and flow situations.

(2) We have developed an algorithm for automated wavelet denoising of the photoacoustic signals. These signals are denoised online using a LabView interface from the detection electronics to a controlling laptop computer.

(3) In addition we have built and tested a two wavelength method using two tunable laser systems to statistically classify photoacoustic event as arising from hemoglobin or melanin. We are currently building a database of photoacoustic signals to improve statistical classification of all events tested in human samples in subsequent years. We have tested the system in accordance with the paradigm delineated in the research plan. In addition, we are conducting a blind study to test whole blood samples that have been spiked with low concentrations of melanoma cells along with control blood samples. We are planning to test 40 samples to determine sensitivity and specificty prior to enrolling human subjects next year.

Update:

In addition to the above progress, we have received notice that our patent claims have been accepted and we expect to receive a notice of allowance on our claims soon. The patent should then be issued early next year. When our initial patent is issued, we will seek angel funding to license the IP to Verapulse, LLC, a company formed by John A. Viator, Ph.D.

Project #:	09-1177
Project Title:	iPrep: Ophthalmic Povidone-Iodine Antiseptic Formulation
Award Amount:	$574,450
Center for Excellence:	Springfield
Lead Investigator:	Dr. Wendell Scott, St. Johns Medical Institute
Collaborators:	Dr. Paul Durham, Missouri State University

Summary:

Endophthalmitis is the inflammation of intraocular cavities of the eye most commonly caused by microbial infection, which can lead to decreased and permanent vision loss. Although the risk of endophthalmitis can never be completely eliminated, taking proper precautions such as pre- and post-surgical antisepsis, aseptic surgical technique, and prophylactic antibiotics can greatly reduce the incidence. An issue of particular concern involving ocular surgery is the best method for disinfection. The eye is highly sensitive to pH, tonicity, viscosity, concentration, and surface tension making it one of the most difficult parts of the body to disinfect.

Povidone-iodine (PVI) is currently used as a topical pre- and postoperative antiseptic for the eye through off-label methods of manually diluting the standard 10% product for the skin with sterile saline to achieve a 5% concentration. The off-label use of the 5% PVI causes local inflammation as well as death to the healthy epithelial cells that provide the covering of the eye. Since the current formulation is also highly acidic, it causes pain and discomfort to the patient when applied to the eye without first using an anesthetic. Their novel formulation of PVI, iPrep, has a neutral pH allowing for direct application to the eye without irritation and a lower concentration of iodine, which significantly reduces damage to healthy epithelial cells while maintaining its excellent antiseptic properties.

Other benefits of iPrep include the inability to develop bacterial resistance, capability to be used repeatedly in all age groups, inexpensive to manufacture, and highly effective against a wide range of infectious agents including Gram positive and negative bacteria, fungi, viruses, protozoa, and in particular, important ocular pathogens: Herpes simplex, adenovirus, HIV, and *Acanthamoeba*. Since the iodine concentration has been optimized and the irritation factors have been eliminated, iPrep can be

extended beyond pre- and post-surgical antisepsis to an over-the-counter eye antiseptic to reduce infections caused by minor cuts and scratches.

Another application of iPrep is for communicable ophthalmic infections such as Trachoma. Trachoma is a bacterial eye infection estimated to affect over 6 million people making it the leading cause of preventable blindness worldwide. Unfortunately, third-world countries are most affected by the debilitating effects of this disease. iPrep is a safe and inexpensive ophthalamic antiseptic that is anticipated to provide a readily accessible therapy which would drastically change the way Trachoma is treated.

The collaboration between St. John's Medical Research Institute and the Center for Biomedical and Life Sciences utilizes the strengths of a well established health care facility and those of higher education expertise to engineer and test iPrep both in animal and human trials. iPrep is ideal for investment by the MLSRB as a medical device according to the FDA United States Code [321] due to the straightforward nature of the transition to commercialization and the potential extensive impact to Springfield and the State of Missouri, as well as national and international communities.

Update: St. John's Medical Research Institute (SJMRI) and Dr. Wendell Scott have continued its progress to produce a novel formulation of povidone iodine specifically designed for use in ophthalmics. This enhanced formulation will allow ocular surgeons to place the product directly into the eye without causing irritation, reducing damage to healthy epithelial cells, and remaining highly effective as an antiseptic.

After thoroughly reviewing the product requirements for efficacy, toxicity, stability, osmolality, and iodine concentration, a formulation has been finalized. SJMRI will be submitting a provisional patent application and will continue stability and quality testing throughout the patent timeline.

Following multiple evaluations of the most appropriate path to receive FDA approval for the product, it was identified that the product will undergo the New Drug Application approval process. Due to funding restrictions relative to the high expenses associated with New Drug Applications, SJMRI is looking to partner with an ophthalmic drug company interested in a co-development relationship. SJMRI is working to identify companies highly specialized and embedded in the ophthalmic market that are interested in working with SJMRI to receive FDA approval and to sell the product.

The infrastructure and resources now available through receipt of this commercialization award, which served as a starting point for commercialization for SJMRI, has led to the successful licensing of two unrelated products developed at SJMRI to two new Missouri start-up companies and there are many more technologies in the SJMRI Research & Development pipeline.

The last remaining work is to conduct a Cytotoxicity study. This work is anticipated to begin in 30 days and take approximately 90 days to run. The goal of the study is to ensure the formulation meets Cytotoxicity parameters established by the FDA.

POVINOL
STERILE OPHTHALMIC
ANTISEPTIC

FY 2008 Life Sciences Research Trust Fund Grant Summary

Total Grant Funding Available in FY2008: $13,100,000

Research Funds Available in FY2008: $10,500,000
Commercialization Funds Available in FY2008: $ 2,600,000

Full Proposals Received -- Research

Centers for Excellence	Requested Research Funds	# of Requested Projects	Awarded Research Funds	# of Awarded Projects	% of Available Funds Awarded
Kansas City	$ 7,116,890	12	$ 2,650,915	5	25.2%
Springfield	$ 1,606,315	3	$ 950,908	2	9.1%
St. Louis	$ 4,210,849	1	$ 2,989,703	1	28.5%
Statewide	$12,962,501.33	18	$ 3,908,474	2	37.2%
Subtotals	**$25,896,555.33**	**34**	**$10,500,000**	**10**	**100%**

Full Proposals Received -- Commercialization

Centers for Excellence	Requested Commercialization Funds	# of Requested Projects	Awarded Commercialization Funds	# of Awarded Projects	% of Available Funds Awarded
Kansas City	$2,559,640	3	$ 325,000	1	12.5%
Springfield	$ 500,000	1	$ 0	0	0%
St. Louis	$1,326,775	1	$ 1,136,719	1	43.7%
Statewide	$4,586,459	4	$ 1,138,281	2	43.8%
Subtotals	**$8,972,874**	**9**	**$2,600,000**	**4**	**100%**

Summary of Grants Awarded in FY2008

Center for Excellence	Total Awards	Total Projects Awarded	Total % of Funds Available
Kansas City	$ 2,975,915	6	22.7%
Springfield	$ 950,908	2	7.3%
St. Louis	$ 4,126,422	2	31.5%
Statewide	$ 5,046,755	4	38.5%
Total	**$13,100,000**	**14**	**100%**

FY2008 Funding Summary – Research

Grant Number	Project Title	Principal Investigator	PI Institution	Grant Category	Funds Awarded
13230	Ultrahigh-Throughput Sequence Profiling of Small RNA in Brachypodium Distachyon, an emerging model for Cereal and Biofuel Crops	Dr. Julia Chekanova	University of Missouri-Kansas City	Plant Science	$558,020
13234	Bone Fracture Repair in Animals Using a New Bond Cement	Dr. David Eick	University of Missouri-Kansas City	Animal Health	$786,998
13238	Evaluation of Candidate Diagnostic Targets for Johne's Disease in Livestock	Dr. Brian Geisbrecht	University of Missouri-Kansas City	Animal Health	$675,000
13243	Grape Polyphenols: Potential for New Commercial Products and Enhanced Plant Health	Dr. Laszlo Kovacs	Missouri State University	Gateway Fund	$897,955
13246	Novel Therapeutic Strategies for the Treatment of Eye Diseases in Animal	Dr. Ashim Mitra	University of Missouri-Kansas City	Animal Health	$312,273
13248	Integrated Program for the Development of Microalgae as Sustainable Resources for Biofuels and Biomaterials	Dr. Paul Nam	Missouri University of Science & Technology	Bioenergy	$526,906
13249	Insect-Deterrent and Antifeedant Properties of Ginkgo Biloba	Dr. Maciej Pszczolkowski	Missouri State University	Gateway Fund	$52,953
13250	Discovery and Utilization of Enzymes for Renewable Biofuels Production	Dr. Himadri Pakrasi	Washington University	Bioenergy	$2,989,703
13254	Identification of Functional Replication and Transcription Linked to Residues for Chromatin Assembly by Histone H3 Proteins in the Corn Smut Ustilago and the Yeast Saccharomyces	Dr. Jakob Waterborg	University of Missouri-Kansas City	Plant Science	$318,624
13321	Advancing Animal and Plant Agricultural Sciences in Missouri	Dr. Marc Linit	University of Missouri-Columbia	Animal, Plant, Environmental Science	$3,381,568
				TOTAL	**$10,500,000**

FY2008 Research Project Summaries

Project #:	13230-2007
Project Title:	Ultrahigh-Throughput Sequence Profiling of Small RNA in Brachypodium Distachyon, an Emerging Model for Cereal and Biofuel Crops
Award Amount:	$558,020
Center for Excellence:	Kansas City
Lead Investigator:	Dr. Julia Chekanova, University of Missouri-Kansas City
Collaborators:	Todd Mockler, Oregon State University

Summary:

Progress in understanding the basic biology and mechanisms of gene function in monocot grasses (the world's predominant grass species), including cereal species cultivated for food and feed, as well as dedicated biofuel crops such as switchgrass, has been severely constrained for many years due to the lack of convenient experimental systems (i.e. model crops). For example, RNA has emerged during the past decade as a key controller of genome function, yet very little information on small RNA function in monocot species is currently available. Brachypodium distachyon (a genetic relative of wheat, barley, and switchgrass) has recently emerged as a premier model for studying how genes function in more genetically complex temperate grasses because of its simple growth requirements, rapid life cycle, small size, and relatively simple "genome," or catalogue of hereditary information. This study will use the model grass, Brachypodium, to fill the significant gap in knowledge that exists for monocot grasses such as wheat, barley, oats, and switchgrass. Ultimately, this new knowledge will benefit Missouri's agriculture and biofuels industry through higher yields for these crops and more efficient energy conversion.

In the first years of funding small RNAs have been firmly established as key regulators of genome function in diverse organisms including plants, yet the biology of small RNAs in monocot plants remains poorly studied. The goal of this proposal is to create a comprehensive catalog of small regulatory RNAs in Brachypodium distachyon, the emerging model system for such agriculturally important species of cereal crops as wheat, corn, rice, barley, as well as for dedicated biofuel crops such as switchgrass, i.e. for the most economically important group of plants. Because small RNA spectra are known to differ between different tissues, stages and growth conditions, they chose to focus this year on leaf, root and stem tissues, as well as on leaves of plants infected with the plant pathogen fungus Magnaporthe grisea. Magnaporthe grisea is the causal agent of rice blast disease, which is the source of tremendous crop losses worldwide.

They have obtained and propagated Brachypodium distachyon line Bd21, established collaboration with the US Fungal Genetics Stock Center that is housed at UMKC, and obtained USDA permit to carry out plant infections with pathogenic fungi. They identified two strains of Magnaporthe grisea (Guy-11 and 70-15) causing infection of Brachypodium distachyon line B21 plants and carried out pilot as well as full scale plant infections. Small RNAs were isolated from three tissue types (leaves, roots, stems) as well as from leaves of plants infected with Magnaporthe grisea. These small RNAs are being used for small RNAs library construction. Solexa deep sequencing of first six small RNA libraries is scheduled for January 2009.

Update:

The goal of this project is to identify the majority of small RNAs in Brachypodium, including those that are cereal-specific and those regulated by abiotic and biotic stress, as well as those that are induced in response to infection by pathogenic fungi.

Small RNAs isolated from total plant and tissue types such as leaves, roots and stems had been used for small RNAs library construction and Solexa deep sequencing. We have also sequenced small RNAs isolated from leaves of plants infected with the plant pathogene fungus Magnaporthe grisea, a causal agent of rice blast disease. Leaves of plants subjected to mock infection were used as a control. In order to be able to subtract Magnaporthe specific small RNAs from infection induced Brachypodium small RNAs we had also sequenced small RNA population from appressoria (fungi hyphal branch which facilitates penetration of the host plant).

Salt, drought and cold stress are among the most frequent and devastating challenges that affect agriculturally important crops. In order to elucidate small RNA-regulated gene circuits under most common stress conditions that crop plants encounter we subjected plants to numerous aboitic and biotic stresses such as heat, cold, drought and salt. Leaves and roots of plants challenged for a different period of time with either heat or cold were used for small RNA library construction, all these libraries had been deep sequenced. We are in a process of collecting tissues from plants subjected to various drought and salt stresses. Analysis of sequenced small RNAs had being initiated.

This project consists of two parts, one experimental and one computational. We expect to finish the experimental part by the end of March 2011. However, the alignment and analysis of the sequence data produced experimentally will require some additional time to be fully completed and published.

Project #:	13234-2007
Project Title:	Bone Fracture Repair in Animals Using a New Bond Cement
Award Amount:	$786,998
Center for Excellence:	Kansas City
Lead Investigator:	Dr. David J. Eick, University of Missouri-Kansas City
Collaborators:	Donna M. Pacicca, The Children's Mercy Hospital

Summary:
Pets and large animals, such as horses, currently benefit from biomaterials (materials used in medical devices that interact with the body) that are used to stabilize and heal bone fractures. Frequently, a bone cement is used to stabilize fractures and prosthetic devices such as those used for hip replacement in dogs with hip dysplasia, a disease that can cause crippling lameness and painful arthritis. The bone cement that is currently used for this purpose is a strong resin (a methacrylate called PMMA) that has several significant drawbacks – it impedes the healing of the bone due to severe toxicity and heat generation and it contracts while solidifying.

The scientists leading this project have developed a silorane-based resin that is superior to PMMA in several important ways. This resin, for example, maintains the same strength as PMMA without contracting or shrinking while drying. It is also less toxic and generates less heat. Additionally, preliminary data suggest this resin actually supports, rather than hinders, bone formation. The results of this study will be the development of composites that have enhanced strength and compatibility with the body. Fillers will be included in the form of hollow microspheres (biodegradable glass) that could be filled with antibiotics or growth factors that induce bone growth and blood vessel formation. Accomplishment of these goals will lead to a commercial application that will improve bone health in both large and small animals.

Update:
We have developed protocols to produce the silorane-based resin for the bone stabilizer/cement. Light-initiation systems have been previously developed for this silorane; however chemical initiation is desired for the proposed application. Chemically initiated polymers do not require an external energy source, such as a curing lamp, to start the initiation process. A suitable chemical-cured/light-cured system, that requires exposure to an ordinary halogen light source for initiation, has been identified. The properties of

this system are still being analyzed. A platinum catalyst has been identified as a potential chemical initiator. Currently, the handling properties achieved with this initiation system are less than ideal, but further optimization should greatly improve these properties. Potential filler particles have been synthesized in a variety of dimensions, geometries and chemistries. Methods have been developed for creating alumina nanorods/whiskers, which are less than a micron in length, and non-alkali glass beads with a diameter of approximately 100 μm. In addition, a surface modification compound for the filler particles has been developed to improve the interaction between filler and silorane resin.

Standards exist for characterizing bone cements; however, the amount of material required for these tests is not compatible with the experimental nature of this study. Furthermore, the application of bone stabilization requires a battery of test characteristics to assess different formulations. Therefore, test methods have been developed, either as modified from current standards (ISO 5833 or ASTM F 451) or developed specifically for the proposed applications, to characterize the following properties: exotherm, handling properties, compressive strength, flexural strength, tensile strength, tensile modulus, and fracture toughness. Properties were first tested in a photoinitiated silorane resin in order to move forward with material testing of the different filler combinations before the chemical cure system was developed. Unfortunately, several of the test methods developed are incompatible with light-initiated systems, either due to light penetration requirements or polymerization speed. Flexural properties and exotherm measurements (including cure time) were collected from the photoinitiated composites.

A model of the effects of the three remaining fillers (two glasses and nanorods) has been developed to predict flexural strength and modulus, polymerization exotherm, and curing time. This model can be used to identify composite formulations that meet specific criteria, such as high flexural modulus and low exotherm. This function of the model was used to choose four composite formulations with properties predicted to be successful in the bone stabilization application. These materials are currently undergoing cytotoxicity screening, before animal testing to assess their ability to stabilization bone fractures.

Studies have been performed comparing the biological response of the silorane resin to bone cement and methacrylate controls. Cytotoxicity has been determined using cell lines from both connective tissue and bone. Initial studies looked at the effect of a small drop of resin on the cells. The silorane resin was shown to have greater biocompatibility than methacrylate resin. These studies led to the question of whether cells could grow directly on silorane resin. An additional set of studies attempted to address this question by growing cells on large disks of material. Microscopic images showed cell viability on the silorane through evidence of cell activity. However, the number of cells growing on the silorane disks was reduced. It was later determined that the lower cell count was due to the reduced ability of the cells to attach to the silorane polymer. All of these studies were conducted using light-cured silorane resins. As mentioned previously, chemically-cured composites are desired for orthopedic applications. One preliminary study with a chemically-cured system produced high cytotoxicity, assumed to be a result of incomplete cure and excessive residual acid from the initiation system. However, the most recent chemical initiation system showed only minimal cell toxicity. Further studies will be conducted to optimize the chemical/light cure systems.

Animal studies have been developed to determine the ability of silorane composites to stabilize bone. Pilot tests were first conducted on bones removed from the animals. Then, studies were conducted on living animals. After introducing a clean-cut fracture to the thigh bone, either neat or filled silorane was applied around the bone creating a 1-mm wide by 1-2 mm thick band of composite material. The flexural strength of the stabilized bones was then determined. These tests have shown that by adding fillers to the silorane resin, the load to failure of the stabilized mouse femora can be greatly increased. Preliminary testing has shown that a mouse femur can be stabilized for up to 4 weeks. While the fractured bone was stabilized with silorane, the mouse only slightly favored the stabilized extremity during the study and radiographs confirmed that the bone was adequately stabilized. Further testing is planned to determine the biological effects of the silorane composite on the fracture healing process.

Project #	13238-2007
Project Title:	Evaluation of Candidate Diagnostic Targets for Johne's Disease in Livestock
Award Amount:	$675,000
Center for Excellence:	Kansas City
Lead Investigator:	Dr. Brian V. Geisbrecht, University of Missouri-Kansas City
Collaborators:	John P. Bannantine, National Animal Disease Center, U.S. Department of Agriculture

Summary:
Johne's disease is a fatal livestock disease that results from a chronic infection of the gut (stomach and intestines) by the widely distributed environmental organism Mycobacterium avium paratuberculosis (MAP). The economic impact of Johne's disease accounts for an estimated loss of $1.5 billion per year to U.S. livestock producers. Johne's disease is widely considered to be one of the most serious issues affecting livestock in Missouri.

In addition to livestock concerns, mounting evidence suggests that milk production from infected animal results in contamination of a large percentage of the nation's dairy supply, since common methods of pasteurization do not destroy MAP. It is therefore essential for both economic and human health related reasons to have adequate methods of diagnosing Johne's disease in cattle as well as methods for testing dairy products for MAP contamination. Unfortunately, affordable and effective tests for this organism and Johne's disease have yet to be developed.

The objective of this proposal is to examine target immunological biomarkers that are predictive of infection of livestock with the environmental microbe Mycobacterium avium paratuberculosis (herein referred to as MAP). The ultimate goal of this research is to lay the foundation for translation of these studies into affordable, field-deployable diagnostics for Johne's disease in multiple agricultural settings. Separately, with the funding period for this award drawing to a close, we are looking to leverage the preliminary data we have gathered so far and to begin exploring other potentially valuable avenues of investigation on related topics.

Update:
While intense research is being conducted to develop faster and more reliable methods for diagnosis of Johne's disease, there are still significant knowledge gaps concerning the molecular pathogenesis of this organism that might aid in vaccine design strategies. Due to the intracellular location of the pathogen during infection, a safe and effective vaccine is likely to be either a live attenuated or possibly even a subunit vaccine. However, in order to produce an effective subunit vaccine, as well as to investigate new biomarkers of infection, new candidate protein antigens must be identified. In the spring of last year, we, along with the laboratory of our collaborator, Dr. John P. Bannantine of the USDA (Ames, IA), published a manuscript describing the characteristics of an extensive MAP recombinant protein set. This work came together by merging our studies at UMKC (funded by the MLSRB) together with those ongoing at the USDA toward a comprehensive large-scale MAP antigen discovery project. All told, 651 MAP proteins were produced in *Escherichia coli*. This set of proteins has provided a unique look into the humoral immune response of MAP during Johne's disease progression, and the published study lays the foundation to identify additional antigens recognized by host immune response at different stages of disease. By evaluating sera from infected cows at the subclinical and clinical stages of infection, this approach may eventually lead to both vaccine protection and diagnostic studies using the identified antigens.

When compared to other bacterial pathogens, a lingering issue that confounds many of overriding scientific goals regarding MAP biology and Johne's disease is that there are comparatively very few reagent systems available. We believe that this extensive recombinant protein repository provides a vital

and powerful tool for proteome- and genome-scale research of this organism. With a resource that enables production of all proteins in the proteome, it becomes possible to analyze the activities of proteins in cell growth, maintenance, regulation, survival and pathogenesis. Additionally, this resource can provide reagents for functional proteomic experiments, including structural studies and defining protein-protein interactions. Finally, tools such as this repository build on genomic information by aiding in the functional annotation of the hundreds of hypothetical proteins. Given all of this, we are confident that this work will result in important advances in understanding MAP and Johne's disease in the future.

As was mentioned in our previous report, a handful of the more attractive Johne's diagnostic targets have been examined in more detail. Among these, we currently have the most data on antigen MAP1272c. This bacterial protein elicits a robust immune response in both subclinical and Johne's positive cattle. It is expressed and localized to the surface of this bacterium, where it, along with several of its homologs, appears to function in remodeling the cell's peptidoglycan layer. Because of this surface-exposed localization, antibodies that recognize this protein should be diagnostic to the presence of the MAP organism. Through our collaboration with Dr. Bannantine at the USDA-ARS (Ames, IA), we have identified two such antibodies (denoted 14C5 and 8G6) that recognize MAP1272c. Immortalized B-cell hybridoma lines for both 14C5 and 8G6 have been single-cell cloned and selected for highest levels of IgG expression. In the past, we felt that the most promising way to proceed with diagnostic development was to engineer single-chain antibodies (scFv) that maintained the properties of the intact IgG. However, despite our best efforts, we have been unable to generate any tangible success in producing such synthetic antibody mimics. Be that as it may, at this point we have only a little more work to do on characterizing the intact monoclonal antibodies before we can both publish our data and submit a new, competitive application for Federal funding to continue development of these diagnostic tools. Briefly, this work includes completing some gene sequencing analysis and quantifying these antibodies' specificities by examining their reactivity towards closely related proteins from the same organism (e.g. MAP1204).

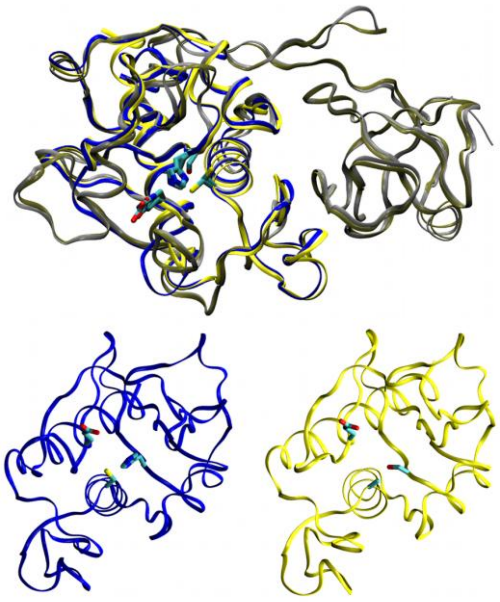


Fig. 1 Structural superimposition of MAP1204 and MAP1272c with two published cyanobacterial NlpC/P60 proteins. Models for *Anabaena variabilis and Nostoc punctiforme* NlpCs, PDB accession codes 2HBW and 2EVR, were aligned using the program VMD. The highly similar *Anabaena* and *Nostoc* structures are colored gray and tan, respectively. MAP1204 has been rendered with a blue backbone, while MAP1272c is yellow in color. The sidechains of the three residues of the catalytic triad are shown for all four proteins as licorice bonds. Both cyanobacterial proteins include an additional amino terminal domain which has been proposed to enhance substrate specificity. Additional domains are not present in a substantial subset of mycobacterial NlpC/P60 proteins, including MAP1204 and 1272c.

Because the majority of MAP gene products have no known function, we have also pursued limited structure-function analysis of those MAP proteins which are the most promising diagnostic targets. This helps to fulfill one of the underlying goals of this research, which is to further our understanding of basic MAP biology. Like all *Eubacteria*, MAP is encapsulated by a protective lattice of crosslinked peptidoglycan, which must be at least partially disassembled during cell division. This is accomplished by three classes of enzymes: glycosidases which cleave bonds between the repeating disaccharide, amidases, which hydrolyze the bond between the peptide moiety and the N-acetylmuramic acid, and finally the NlpC/P60 endopeptidases, which cleave the interchain bonds. Along these lines, we have previously described the atomic resolution crystal structures of the two proteins (MAP1272c and MAP1204) mentioned above. From this, we made the surprising observation that MAP1272c does not have a functional catalytic core. It is clear from the structure of MAP1272c that the residues required for hydrolysis are absent, strongly suggesting a role as a binding protein or receptor for the

peptide moiety of peptidoglycan. While the catalytic triad is present in MAP 1204, other residues appear to partly occlude access to the catalytic site, suggesting an unique specificity compared to known NlpC/P60 enzymes. This information can be found in **Fig. 1**, which appears to the above.

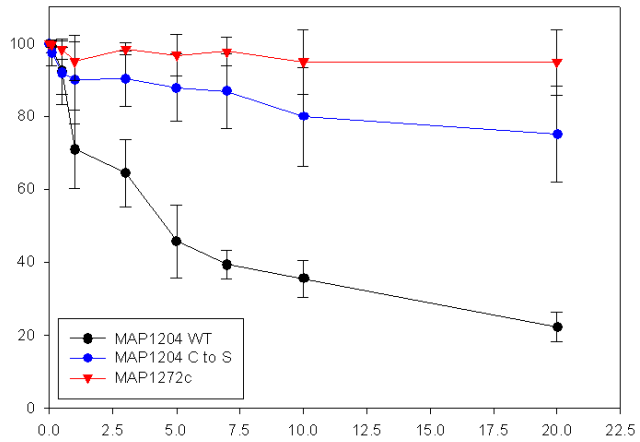


Fig. 2. Incubation with MAP1204, but not MAP1272c, results significantly reduced survival of *B. subtilis* cells on non-selective media. Concentration of protein is shown on the X-axis (uM units), while per cent cell survival is plotted on the Y-axis. These results demonstrate that MAP1204 is a functional NlpC/P60 amidase, while MAP1272c lacks a similar activity, and they directly support the structural analysis described above and presented in **Fig.1.**

To test directly the predictions of our structural analysis, we needed to establish a functional assay for NlpC/P60 activity. However, using synthetic mimics of the unusual substrates found in bacterial cell peptidoglycan layers for *in vitro* assays is practically cost-prohibitive: only a few mg cost several thousand dollars! As a result, we initiated a collaboration with the laboratory of Dr. William D. Picking, who is the head of the Department of Microbiology and Molecular Genetics at Oklahoma State University (Stillwater, OK). Dr. Picking is an experienced microbial biochemist, who helped us design a suitable assay for bacterial survival in the presence of exogenous quantities of these putative peptidoglycan remodeling enzymes. The concept of this assay is straightforward, in that the integrity of the bacterial cell should be compromised in the presence of inappropriate NlpC/P60 activity, and that integrity of the cell wall is essential for cellular viability. The results of this study are shown in **Fig. 2**. This work establishes MAP1204 as an active NlpC/P60 protein, and demonstrates that mutation of the proposed catalytic Cys residue greatly diminishes the enzyme's activity. Finally, these results also confirm the notion that MAP1272c lacks the enzymatic activity characteristic of all other members of the NlpC/P60 family. To our knowledge this is the first ever description of such a protein. Altogether, we feel that this work stands as a significant contribution to our understanding of important class of bacterial wall remodeling enzymes. We are currently drafting a manuscript describing these data for submission to the *Journal of Biological Chemistry*.

It is significant that serendipitous and engineered deletion of related NlpC/P60 protein-encoding genes has been shown to by others to reduce both pathogenicity and vegetative growth of bacteria. As a consequence, members of this family of enzymes are considered to be attractive targets for next-generation antibiotic development. Since MAP is resistant to all front-line anti-mycobacterial antibiotics, novel approaches must be explored in treating infections with this organism. Furthermore, since NlpC/P60 proteins are found in all *Eubacteria*, any compounds that are capable of inhibiting these enzymes have the potential to be developed into new, broad-spectrum antibiotics. In this regard, it has not escaped our notice that our structural information, along with this functional assay, may represent a potentially useful platform for screening for such compounds. We are currently planning to conduct some limited screening of compound libraries, by using other resources available to the laboratory. If interesting results should arise, these would be vital preliminary data for future grant applications.

Project #:	13243-2007
Project Title:	Grape Polyphenols: Potential for New Commercial Products and Enhanced Plant Health
Award Amount:	$897,955
Center for Excellence:	Springfield
Lead Investigator:	Dr. Laszlo Kovacs, Missouri State University
Collaborators:	Wenping Qiu, Missouri State University; Richard Biagioni, Missouri State University; Paul Durham, Missouri State University; Daniel Schachtman, Donald Danforth Plant Science Center; and Oliver Yu, Donald Danforth Plant Science Center

Summary:
Grapes synthesize a plethora of polyphenolic compounds (such as tannins and lignins), many of which improve the health of both the plant and the human who consumes it or its product (e.g. wine or juice). This study will focus on two varieties, Norton and Cabernet Sauvignon, the former of which is the most prominent wine grape in Missouri. The project scientists will work to identify the individual compounds, or classes of compounds, that provide the health benefits provided by grapes and grape products. The resulting information will lead to the development of novel high-value grape products, such as wines, food supplements, and herbal condiments with scientifically-proven dietary value. This research project will also identify genes that direct polyphenol synthesis in the berries that respond most effectively to plant pathogens or diseases. Knowledge acquired from these studies will lead to healthier fruit products and hardier plants, resulting in improved human health and lower fungicide usage in Missouri vineyards.

In the first year of funding they collected berry tissue (seed and skin) samples from two different grapevine varieties (Norton and Cabernet Sauvignon) at defined developmental stages all through the growing season. They are extracting nucleic acids from these tissues to begin studying genes of enzymes that synthesize polyphenolic compounds in the berry. In addition, they began collecting information on the key regulators of the flavonoid pathway, the route by which the major polyphenolic compounds are synthesized in grape cells. Twelve genes of these regulators have also been cloned and are now being introduced into grape roots to determine how they influence flavonoid biosynthesis.

A comparative analysis of the polyphenolic compounds themselves is underway in both Norton and Cabernet Sauvignon. More than ten novel anthocyanins (color-producing polyphenols) that are specific to Norton were already identified at the level of molecular structure for the first time. Chromatographic studies also are underway to compare the amounts of seed polyphenols as they accumulate during the course of berry development. In bioactivity studies, they have found that polyphenols extracted from the seed possess considerably higher anti-inflammatory activity than those from the berry skin. Seeds also contain higher quantities of these compounds, with maximal quantities measured at the developmental stage at which the berry starts to change color and ripen (veraison).

Currently, large-scale experiments are underway in which seed extracts are fractionated and the anti-inflammatory activity of the various fractions are tested. Work is also in progress to determine how long the polymers are that accumulate in the seed and in the berry skin, with results already showing that proanthcyanidins form shorter polymers in the seed than in the berry skin. Polymer length may be one of the characteristics that are important in determining the anti-inflammatory potential on these compounds.

Update:
The Final Report on this research program will provide detailed results in three major areas: (1) the identification of a grape seed-derived non-polar compound and polyphenols and their influence on chronic inflammation in nerve cells; (2) the regulation of organ development and secondary metabolism

in grape seeds; and (3) metabolism- and defense-related gene regulation in the berry skin of the Missouri grape variety Norton.

1) We have isolated a non-polar compound in grape seeds that consistently lowered the level of the inflammatory neuropeptide clacitonin gene-related protein (CGRP). The data indicated that this compound has the potential to reduce chronic pain. This compound is novel as a grape seed metabolite, but had been previously identified in a medicinal herb. Importantly, the concentration of this compound is considerably higher in grape seeds than in the herb.
   We also confirmed and extended previous findings that grape seed-derived polyphenols are able reduce the intensity of chronic inflammatory events in nerve cells. Using a rat model, we were able to show that consumption of a diet rich in grape seed polyphenols markedly increased the levels of the anti-inflammatory protein phosphatase MKP-1 in both the trigeminal ganglion and the trigeminal nucleus caudalis. Further, CGRP levels were lowered by these compounds. This suggests that the incorporation of grape polyphenols in the diet will reduced the sensitization of peripheral or central nervous system in humans also. This finding has implications for the prevention of chronic inflammatory disease such as migraine. Finally, we determined that polyphenols extracted from early-stage seeds are more effective at modulating the inflammatory response than polyphenols extracted from late-stage seeds.

2) We determined that a critical phase of grape seed development, termed transition, coincides with a sudden increase in abscisic acid levels (ABA). We also showed that transition itself was not triggered by this ABA burst alone, because the developmental processes leading to the transition phase were in place prior to the increase in ABA concentration. In the area of polyphenol biosynthesis, we provided data to explain in Norton grape the relatively low levels of tannins, a key enological characteristic of this variety. We showed that Norton accumulates considerably lower levels of soluble tannins than the well-known world variety Cabernet Sauvignon. Furthermore, soluble tannin levels dropped precipitously during storage reserve accumulation in Norton seeds, but remained relatively unchanged in Cabernet Sauvignon. Concomitantly, the levels of insoluble (oxidatively cross-linked) tannins increased in Norton, reaching higher levels in mature seeds of Norton than in Cabernet Sauvignon. Seeds of the two varieties also differed in the assortment flavan-3-ol compounds. These findings are intriguing in light of our results that Norton seed extracts were more potent modulators of the inflammatory response than Cabernet seed extracts.

3) We showed that berry skin in these two varieties had strikingly different anthocyanin composition. Some of these differences were expected, because Norton berries are known to accumulate diglucosides of anthocyanins whereas Cabernet Sauvignon only mono-glucosides. Interestingly, however, we identified ten compounds that were present only in Cabernet Sauvignon berry skin extracts (see Figure 1 below). This differential anthocyanin composition was reflected by differential expression of structural and regulatory genes in the flavonoid biosynthesis pathway. Our gene expression data also demonstrated that certain transcription factors play a more prominent role in the regulation flavonoid synthesis in Norton berries (MYBPA1 and MYB5A), whereas others play a more prominent role in Cabernet Sauvignon berries (MYBPA2 and MYB5B). We found that many pathogen defense-related genes were expressed more abundantly in Norton than in Cabernet Sauvignon berry skin, which partially explains the exceptionally high disease resistance of Norton grape. Most notably, stilbene synthases and pathogenesis related protein-1 remained at very high levels throughout the ripening process in Norton.

We are currently in the process of publishing some of these data in the scientific literature. Results that have potential for commercialization are being considered for further translational research.

Project #:	13246-2007
Project Title:	Novel Therapeutic Strategies for the Treatment of Eye Diseases in Animal
Award Amount:	$312,273
Center for Excellence:	Kansas City
Lead Investigator:	Dr. Ashim K. Mitra, University of Missouri-Kansas City

Summary:
Topical administration is the most preferred and convenient route for treatment of veterinary eye diseases. However, drug levels absorbed by the eye for most topically applied drugs are less than one percent of the applied dose. Obviously this is an inefficient treatment method. The objective of this research project is to develop drug delivery strategies to significantly improve eye absorption of topically applied veterinary drugs such as erythromycin, prednisolone, acyclovir and bimatoprost for the treatment of bacterial keratitis, inflammations, viral corneal keratitis, and glaucoma. With these grant funds, scientists will continue research on a drug that, when added to eye medicine, results in greater solubility and eye absorption. In addition, a drug is being tested that uses microscopic particles suspended in a gel solution to increase the time the substance resides on the eye and sustains the release of the drug. This strategy allows a single treatment application for one week therapy, causing much higher efficacy of the applied medication, particularly in companion animals.

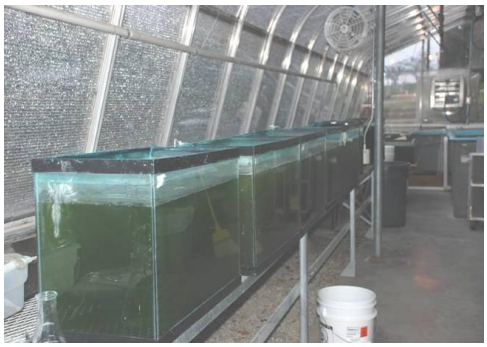
Update:
The broad, long-term objective of this grant application was to develop drug delivery strategies to improve ocular absorption of topically applied veterinary drugs such as erythromycin, prednisolone, acyclovir (ACV) and bimatoprost for the treatment of bacterial keratitis, inflammations and viral corneal keratitis respectively. We have already reported to the MLSRB that erythromycin and bimatoprost have been replaced with ganciclovir (GCV) and gatifloxacin respectively due to higher clinical significance to veterinary health sciences. The first specific aim involves the synthesis and characterization of the sterioisomeric prodrugs of ACV, GCV and prednisolone. This goal has been completed. The prodrugs have been characterized for their interaction with peptide transporters. Bioreversion and stability studies for ACV and GCV prodrugs have been carried out. The pharmacokinetic parameters of these prodrugs have also been studied in ocular tissues. We have screened gatifloxacin for its affinity towards the efflux transporters (P-glycoprotein, Multidrug resistance associated protein and Breast cancer resistance protein). Synthesis of sterioisomeric prodrugs of gatifloxacin is in progress.

We have also prepared and characterized nanoparticles of ACV, GCV and Prednisolone along with their prodrugs and generated release profiles. The prodrugs of prednisolone suffer from poor entrapment efficiency in nanoparticles hence we are using prodrugs of dexamethasone as model substrates to solve this problem. We are using a unique hydrophobic ion pairing with complex technique to enhance the entrapment efficiency of these prodrugs and the results from these studies will be applied to prednisolone prodrugs. We have synthesized and characterized a novel pentablock (PB) polymeric material made of multipolymer blocks like polyglycolide, polyethylene glycol and polycaprolactone (every block of PB polymer is FDA approved for human use). Since polyglycolide has a faster biodegradation profile relative to polycaprolactone, varying the ratios of these two blocks polymers in the PB polymers can optimize the long term release of these sterioisomeric prodrugs in the eye. We are planning also planning to utilize this novel PB polymer (US patent in preparation) to prepare and characterize nanoparticles. Prodrug entrapment efficiency, particle size, and release profiles will be optimized by adjusting polymeric block ratios of PB polymer. Nano particles containing ACV, GCV, prednisolone, gatifloxacin and their prodrugs will be soon be ready for testing in rabbits. A suitable long term delivery system will be available for testing in animals (Cats and Dogs) by mid 2010.

The funds allotted by the State of Missouri for this grant proposal have been used to acquire a high performance liquid chromatography system along with various cell culture material and reagents required to accomplish the tasks proposed in the project.

Project #:	13248-2007
Project Title:	Integrated Program for the Development of Microalgae as Sustainable Resources for Biofuels and Biomaterials
Award Amount:	$526,906
Center for Excellence:	Statewide
Lead Investigator:	Dr. Paul Nam, Missouri University of Science and Technology
Collaborators:	Keesoo Lee, Lincoln University; Virgil Flanigan, Missouri University of Science and Technology; and Fabio Rindi, University of Alabama

Summary:



Aquatic microalgae are photosynthetic microorganisms that have great potential to be the solution to growing energy and environmental challenges. Multidisciplinary collaborative research is conducted to develop economically-feasible microalgal biotechnologies that utilize carbon dioxide and wastewater as nutrient sources and yield biomass that can be converted to biofuels and other bioproducts. The major focuses of the research program are: (1) identification of high yielding, hardy, pest resistant microalgae strains; (2) development of economically-viable, commercial-scale algae cultivation/harvesting systems that mass produce algal biomass and abate carbon dioxide and wastewater; (3) development of an effective system for extracting oil from wet algae and converting to biodiesel; (4) testing methods for fermenting algal carbohydrates into ethanol; and (5) proof of a concept for the self-supported system that integrates the microalgae cultivation/harvesting processes with the bio-refinery which is dedicated to algae-based biofuels and bioproducts.

Update:

In 2010 the flue gas carbon dioxide from a 60 MW coal-fired power plant was used to cultivate the microalgae in five deep circular ponds. The effect of flue gas without any desulfurization on microalgae growth and biomass production was evaluated during the summer months.

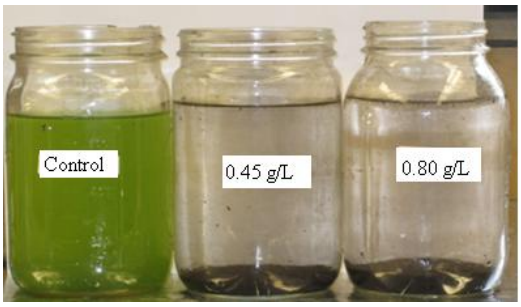
Table: Microalgal biomass productivity of the pilot-scale deep pond injected with the flue gas.

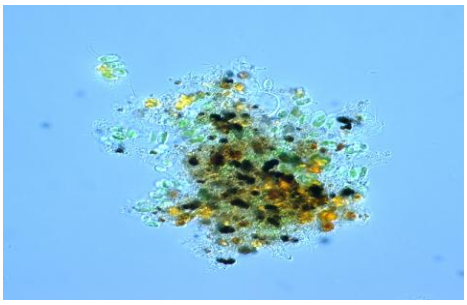
Month	pH	Water temp (ºC)	Light (Lux)	$g/m^2/day$	g/l/day	CO_2 fixation rate
May	9.4	23.9	966	11.4	0.031	0.05
July	8.8	24.0	1106	8.2	0.022	0.04
August	8.6	30.0	1139	12.0	0.032	0.05
October	8.6	16.2	1099	6.3	0.017	0.03

Utilization of the wastewater nutrients for microalgae cultivation was investigated. The results showed the potential of using microalgae for bioremediation of wastewater to reduce nitrogen and phosphorus. Since the prevalence of herbicides such as atrazine in agricultural runoff can present a problem, the maximum tolerance

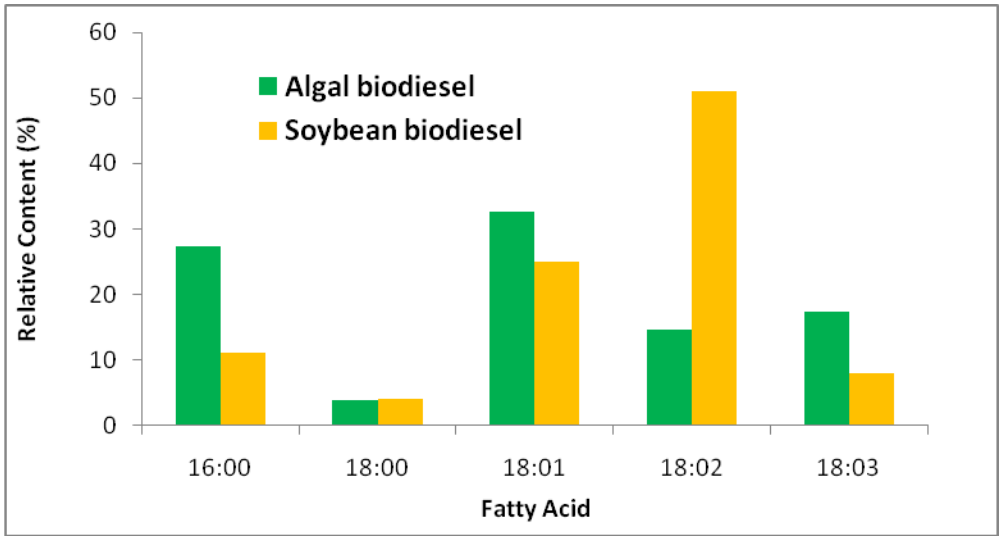
level of atrazine in wastewater for growing algae was determined. The ability of microalgae to use the soluble carbonate salts for their growth was examined as a way to capture and utilize carbon dioxide from power plants if the algae cultivation ponds are located in the distance.

Harvesting microalgae from the cultivation ponds has been a major hurdle. The conventional harvesting methods such as centrifugation and filtration are time and labor intensive processes. Gravimetric settlement with the aid of cationic flocculants is the most widely employed technique, but suffers from the difficulty in recovering microalgae flocs. Furthermore, recycling of these flocculants after the harvesting process is not possible. A novel and efficient process is developed that involves the flocculation of microalgae with specially synthesized particles to efficiently settle the microalgae, easy removal of flocs, and the recycling of the separated flocculant particles.





The catalyst-free transesterification of oil (triglycerides) in supercritical methanol provided a new way of producing biodiesel. High reaction temperature and pressure helped to accelerate the transesterification without the catalyst because the supercritical methanol becomes non-polar and has enhanced contact with oil. Since the supercritical transesterification is carried out without a traditional acid/base catalyst and does not produce any saponified byproducts, the recovery of pure biodiesel product becomes much simpler. After the reaction, the products readily separated into two phases of biodiesel and glycerol.



Project #:	13249-2007
Project Title:	Insect-Deterrent and Antifeedant Properties of Ginkgo Biloba
Award Amount:	$52,953
Center for Excellence:	Springfield
Lead Investigator:	Dr. Maciej Pszczolkowski, Missouri State University

Summary:
The goal of this research project is to develop a novel pest control strategy targeting internal fruit feeding insects. Codling moth, the most significant apple pest problem for Missouri growers, infests the fruit as "neonate," or newborn, larvae within twelve hours after hatching from the egg and stays inside the fruit until its development is complete. Recently advocated Codling moth control measures that target adults are only at an early stage of development in Missouri and may not be effective enough to provide satisfactory control measures. Historically, broad-spectrum contact insecticides have been used to target larvae just after hatch. However, these insecticides pose a severe risk to human health, negatively impact the natural environment, and will soon be banned by the federal "Food Quality and Protection Act." If no alternatives are commercially available after this pesticide is banned, it is estimated that Missouri apple growers will lose at least $5.2 million in annual income.

It is already known that ethanolic extracts from Ginko biloba (a Chinese tree that is exceptionally resistant to insect pests) prevents apple infestation by Codling moth neonates. This study will determine which Ginko substances discourage neonates from infesting fruit. By identifying these substances, the study will open a new avenue for Codling moth control with substances derived from an herb which has been known to be beneficial to human health, thereby helping to reduce potential human health risks and minimize adverse environmental effects from currently used Codling moth control measures. The study will be undertaken in cooperation with an internationally recognized expert on modifying Codling moth larvae behavior with plant extracts. The results will be licensed to a Missouri-based company which produces chemicals for agricultural applications.

This two-year project aims to identify the components of Ginkgo biloba that have antifeedant and deterrent properties toward the major cosmopolitan pest of apples, codling moth, Cydia pomonella.

During the first year, the PI and co-workers established a season-independent choice assay for testing Ginkgo extracts. Methodology of this assay has been published in a peer-reviewed research paper, which is scheduled to appear in Journal of Entomological Society of British Columbia by the end of December 2008. Moreover, they partitioned Ginkgo extracts into five fractions, each containing different class of chemicals that are found in Ginkgo foliage. Three classes of Ginkgo constituents were inactive in their assays. One class is still being tested. Yet another class of Ginkgo constituents has deterrent properties and prevents fruit infestation by codling moth larvae in dose- dependent manner. They are in the process of identifying the deterrent chemical compound in this fraction. Their results pave the way to implementation of strategies of codling moth control with plant derivatives, so called secondary metabolites. These may be synthesized on a larger scale and used for sprays against codling moth larvae. Alternatively, their chemistry may suggest how codling moth- resistant apple varieties could be rationally designed.

Update:
The potential of Ginkgo biloba, and its synthetic metabolites for preventing apple feeding and infestation by neonate larvae of Cydia pomonella was explored and a new methodology of testing and extraction was established. Experiments with crude extracts indicated that deterrent constituents of Ginkgo are present among alkylphenols, terpene trilactones and flavonol glycosides. Further experiments with eleven Ginkgo synthetic metabolites of medical importance indicated that three out of these chemicals have feeding deterrent properties. Substance A prevented fruit infestation at concentrations as low as 1 mg/ml,

Substance B had deterrent effects as low as 0.1 mg/ml, and Substance C had deterrent effects at 10 mg/ml. Substances A, B, and C may be used for preventing apple infestations by the codling moth either as sprayable formulations or by genetically modifying the apple. Further, flavonol glycosides generally promoted fruit infestation by codling moth neonates. Neither alkyphenol, terpene trilactone, nor flavonol glycoside fractions had toxic properties against the codling moth. Neither Substances A, B, or C had toxic properties against the codling moth. Our research is the first report showing that Ginkgo constituents influence fruit infestation behavior and have potential applications in fruit protection.

Project #:	13250-2007
Project Title:	Discovery and Utilization of Enzymes for Renewable Biofuels Production
Award Amount:	$2,989,703
Center for Excellence:	St. Louis
Lead Investigator:	Dr. Himadri Pakrasi, Washington University
Collaborators:	Largus (Lars) Angenent, Washington University; Rajeev (Reggie) Aurora, St. Louis University; Richard Axelbaum, Washington University; Roger N. Beachy, Donald Danforth Plant Science Center; Pratim Biswas, Washington University; Robert Blankenship, Washington University; Jeffrey I. Gordon, Washington University; Tuan-Hua David Ho, Washington University; Monty Kerley, University of Missouri-Columbia; Shelley Minteer, Saint Louis University; Ralph Quatrano, Washington University; Monica Schmidt, Donald Danforth Plant Science Center;Thomas Smith, Donald Danforth Plant Science Center; Gary Stacey, University of Missouri-Columbia;Teresa Thiel, University of Missouri-St. Louis; Xuemin (Sam) Wang, University of Missouri-St. Louis; Dong Xu, University of Missouri Columbia; Oliver Yu, Donald Danforth Plant Science Center; and Zhanyuan Zhang, University of Missouri- Columbia

Summary:
This proposal includes a comprehensive set of biofuels related research projects by members of the Missouri Biofuel Research Consortium in St. Louis, a group of 20 world class plant scientists. The projects focus on three areas: improving the efficiency of transforming biological materials into energy, enhancing the reliability and cost effectiveness of biofuels, and increasing the efficiency of transforming sunlight into energy via biological materials (plants and algae). While fossil fuels will remain a critical fuel for energy generation in the foreseeable future, bio-derived fuels will be an important component of our regional and national energy portfolio. Availability of cheap, abundant energy is imperative for the economic prosperity and national security of any country. This proposal will help biofuels transition from a boutique to a primary energy source, improving both of these important ends.

Update:
The grant funds were used to purchase several pieces of equipment that are utilized by multiple researchers at Washington University for metabolite measurement for biofuel production. Saint Louis University was also able to purchase a Synergy 4 Multi-Detection Reader to screen for the cellulasas enzyme. This equipment has greatly enhanced the biofuels research capacity at Washington University.

Funding was also used to support biofuels research being conducted at the International Center for Advanced Renewable Energy & Sustainability (I-CARES). Over 23 scholarly publications, numerous presentations and at least six research projects can be attributed to this funding.

ALGAE GROUP

- Biodiesel is an alternative transportation fuel that is currently made from vegetable oils by transesterification using methanol or ethanol, which are derived from fossil fuels. Biodiesel, or methyl- and ethyl- esters of fatty acids, has recently been synthesized in *E. coli* by the expression of three genes. Investigators at the *University of Missouri – St. Louis* have modified the three genes needed for FAEE (fatty acid ethyl esters) production so that the genes necessary for biodiesel production are expressed under the control of the strong inducible *nifH* promoter. This

promoter functions well in specific cells in cyanobacteria called heterocysts, and already produce long chain lipids that might serve as a substrate for production of biodiesel.

- To identify cellular factors that enhance oil and biomass production, investigators at the *University of Missouri – St. Louis* have also been identifying genes that regulate storage lipid synthesis and accumulation. Several candidate genes have been found to be involved in storage oil accumulation in seeds. Investigators are using *Camelina sativa* as a model to test the function of these genes in oil production. Camelina is an emerging new oil crop that requires low input of nutrients and water, has a short life cycle (90 days from seeds to seeds), and is easily transformed. Two genes, a Myb transcriptional factor and a phospholipase D, have been placed under the control of a seed specificpromoter.
- Transgenic plants harboring the gene of interest have been obtained, as confirmed by PCR and immunoblotting.
- Using Trust Fund funds, a GC-MS (Gas chromatography-mass spectrometry) facility has been established at *Washington University* for metabolite concentration and isotopomer distribution measurement. Investigators have characterized *Thermoanaerobacter sp. strain X514* and discovered a novel enzyme which can be utilized for butanol synthesis. Meanwhile, the impacts of carbon and nutrient sources on the CO2 fixation in *Roseobacter denitrificans* OCh114 has been examined, which reveals a unique CO2 fixation pathway without using Calvin Cycle.
- The LSTF funds have been used by investigators at *Washington University* for the development of an algal system that produces high level of biohydrogen, a clean source of energy. In this system, sunlight and CO2 are directly used to first capture carbon in the form of glycogen, which is then, in turn, converted inside the cell factory to hydrogen. A recent publication in *Nature Communications* details cyanobacteria *Cyanothece* 51142's unique biohydrogen production process.

ENZYME GROUP

- A central challenge for the production of biofuel using lignocellulose is to identify enzymes, cellulases, that can efficiently break down the cellulose into sugars that can be easily used by an organism. However, no simple inexpensive assays currently exist that can be used for identifying, screening and optimizing cellulases that can digest celluloses from different sources (e.g. Corn,hardwoods, grasses etc.). To that end, investigators at *Saint Louis University* have developed a sensitive, high throughput (96 or 384 well plates), robust, and rapid (30 minutes) fluorescence assay for cellulases that uses the dye Congo red. A manuscript describing this assay, and a patent for the assay are currently being prepared.
- Today, butanol is primarily used as an industrial solvent, however, butanol can also be used as a fuel in present transportation and as a replacement for the use of gasoline and ethanol. To eliminate the problems associated with butanol toxicity to the microbes that can be used to produce butanol and specific product production, *Saint Louis University* investigators are proposing an enzymatic bioreactor by which pyruvate intermediates will be enzymatically converted to butanol. By varying the growth conditions along with cell lysis protocols, investigators were able to increase the NADHdependent butanol dehydrogenase activity in crude extract by 605.3 fold. *Washington University* investigators have confirmed that two inducible promoters are available for use for tissue growth of moss. A secretion sequence will be tested and confirmed in a working sixliter bioreactor in a designated lighted growth chamber for biomass accumulation. Methods to improve production of transgenic protein have been identified, and production has increase by at least four-fold over the last six months. Lab members amplified tissue to check protein accumulation and evaluate the specific activity of these enzymes for use in biomass degradation
- In order to reduce lignin levels during or before full maturation, investigators at the *Danforth Plant Science Center* has generated transgenic Arabidopsis plants containing genes that confer constitutive or inducible silencing of genes that play a key role in lignin biosynthesis. Results

suggest that it will be possible to alter lignocellulose composition in some plants without affecting normal growth and development.

- Investigators at the *University of Missouri – Columbia* worked to identify enzymes capable of degrading plant cell wall polysaccharides into fermentable sugars. It is apparent that for this to be economically feasible, such enzymes should be produced in the cellulosic feedstocks themselves. In theory, such enzymes could be introduced into cellulosic feedstock prior to the pretreatment that is required. However, since pretreatment typically involves elevated temperature and a range of pH, thermostable and pH-stable enzymes are required. The alternative that is being proposed and examined is to produce (in feedstock) the cell wall-degrading enzymes, and then to integrate the enzymes into existing production practices, downstream of the pretreatment step.
- Also at the *University of Missouri – Columbia*, investigators have further developed MUFOLD, a software package, for predicting tertiary structure from a protein sequence. In order to improve the efficiency of bioenergy, it is often important to characterize and design related genes through their protein structures. MUFOLD demonstrated proof of principles in the 2008 community-wide experiment for protein structure prediction (CASP8), and was ranked top 4 in the template-free prediction category among 81 teams.
- Studies at the *Danforth Plant Science Center* also elucidated how the enzyme SusG works with other membrane components to break down and import starch for fermentation. SusG is an a-amylase and part of a large protein complex on the outer surface of the bacterial cell and plays a major role in carbohydrate acquisition by the animal gut microbiota.

Project #:	13254-2007
Project Title:	Identification of Functional Replication and Transcription Linked to Residues for Chromatin Assembly by Histone H3 Proteins in the Corn Smut Ustilago and the Yeast Saccharomyces
Award Amount:	$318,624
Center for Excellence:	Kansas City
Lead Investigator:	Dr. Jakob H. Waterborg, University of Missouri-Kansas City

Summary:
Fungi are often serious plant pathogens which threaten and diminish agricultural crops. In order to control fungi related diseases better, and to learn how one can directly interfere with or change fungal actions, control of gene expression must be better understood. This study uses Corn Smut, a fungal disease that infects corn, as a model. It seeks to understand the role specific histones (spools around which DNA winds) play in growth and development of the fungus. This basic research could eventually lead to applications to guard corn and other important cash crops from fungal diseases.

Update:
The central methodology for Specific Aim 1, i.e. the homologous recombination of histone H3 variant genes by knock-out, knock-in and mutated histone H3 gene cassettes has been accomplished. It is now in continuous use by Postdoctoral Research Associate Anju Verma, Ph.D. to obtain different combinations of primary histone H3 variant coding sequences (and modifications thereof), distinct promoters and selectable markers in the U.maydis genome. We have started a systematic phenotypic analysis of the synthetic mutants obtained with this methodology, evaluating comparisons of gene sequences and protein expression variations, with the help of new graduate students in their training. Phenotypic observations include the rate of cell proliferation and its linkage to the replication-coupled H3 variant; cell survival in stationary and hydroxyurea-arrested cells and its linkage to the replication-independent, transcription-coupled and constitutively expressed replacement H3.1 variant. Selectivity of histone chaperones for the RC H3.2 and RI H3.1 variant has been detected in vivo and experiments are underway to confirm these observations in vitro. The formal proof of the cell cycle dependence of the H3.2 variant gene U2 and of the replacement function of the H3.1 protein in transcriptionally active chromatin has been prepared for submission to J. Biol. Chemistry. The evolutionary analysis underlying the prediction of the unique H3

variant gene features in U.maydis, and their relationship with the single RI-type histone H3 of S.cerevisiae is in the process of publication in BMC Evolutionary Biology.

As discovered in 2009, a unique form of newly synthesized histone H3 in fungi, including U. maydis and S. cerevisiae, can be separated from mature bulk histone H3 by reversed-phase hplc. Progress is being made, including through the use of diverse genomic transformants for H3 variant genes and their promoters, to study the factors involved in its formation and maturation. Conditions are being discovered that lead to depletion or accelerated maturation to mature nucleosomes and others that lead to the apparent transient accumulation of new H3 forms in nuclei. New collaborations are being sought with yeast molecular biologists with interests in nucleosome assembly pathways to explore the complexes in which these new H3 forms exists and in which way their maturation into mature chromatin is dependent on a variety of known yeast chaperones.

The identification of the basis for the separation of new from mature H3 has now been established. Initially it was thought that high methylation levels of lysines and possibly arginines are responsible. However, through AUT gel analysis, western blotting of separated H3 protein preparations for a range of post-translational acetylations, proteolytic fragmentation followed by hplc analysis, it has become clear that acetylation is responsible. New H3 is clearly much higher acetylated (~2.5 acetylated lysines per molecule in both yeast and smut) than mature H3 (1+ acetylated lysines per protein). Mass spectrometry analysis of new and mature H3 V8 peptides has identified that a single lysine, K56, is quantitatively acetylated in yeast and smut new H3, irrespective of the histone variant character in U.maydis. With this lead, an alanine scanning library of yeast H3 for all lysines and arginines was used (a gift from A. Shilatifard, Stowers Institute) to evaluate the production of the unique new H3 hplc peak. This confirmed that only Lysine 56 in histone H3 was responsible for the delayed hplc elution. This will focus the maturation process analysis on chaperones like yeast Rtt106 which have been shown to be K56ac-dependent. This library has revealed that a large number of lysines and arginines, many more than identified in prior studies, likely through their acetylation and/or methylation, affect the synthesis and post-translational modification state of newly synthesized and matured histone H3 protein. This was determined by AUT gel analysis for acetylation and by tritiated methionine fluorography for methylation. These results will be presented at the West Coast Chromatin Meeting in december 2010. It is anticipated that these data will lead to new research directions, likely in collaborations with other investigators. The full M/S analysis of this system for U.maydis and S.cerevisiae is nearing completion. It is the last major data component required to complete a manuscript that is in preparation for J. Biol. Chemistry to describe this new information.

Project #:	13321-2007
Project Title:	Advancing Animal and Plant Agricultural Sciences in Missouri
Award Amount:	$3,381,568
Center for Excellence:	Statewide
Lead Investigator:	Dr. Marc J. Linit, University of Missouri-Columbia
Collaborators:	Rod Geisert, University of Missouri-Columbia; Jack Jones, University of Missouri-Columbia; Rob Kallenback, University of Missouri-Columbia; Monty Kerley, University of Missouri-Columbia; Scott Peck, University of Missouri-Columbia; Keith Striegler, University of Missouri-Columbia; Jinglu Tan, University of Missouri-Columbia; Jerry Taylor, University of Missouri-Columbia; Jay Thelen, University of Missouri-Columbia; John Walker, University of Missouri-Columbia; Wenping Qiu, Missouri State University, Mountain Grove

Summary:
This University of Missouri proposal focuses on strategic investments across a broad spectrum of research stages – basic, transitional, applied – in order to enhance Missouri's position as a national leader in the agricultural sciences. These research projects will focus on both animal and plant science with a particular emphasis on building instrumentation and research equipment capacity, which is a major factor in attracting federal research dollars and, of course, serves as the tool that allows research scientists to develop new technologies. Specific projects will focus on measuring agriculture impacts on Missouri streams, developing livestock odor abatement strategies, improving feed efficiency for pasture-based dairy and beef cattle operations, and extensive study of plant genetics and proteins to improve crop yield. Findings from this research will not only enhance animal and plant productivity, but improve citizens' quality of life while contributing to Missouri economic development efforts.

The Gateway Project consists of nine subprojects as outlined below:

Update:

Genomics

Principal Investigator: Dr. Jerry Taylor

The goal of this subproject was to acquire an Illumina Genome Analyzer "next-generation" sequencing instrument, recruit a technician to support the varied applications of the instrument and to purchase sufficient preliminary reagents to bring the instrument on-line for use by the Missouri plant and animal genome research community. We were also able to leverage the LSTF grant award to secure a discount on the instrument by simultaneously purchasing an Illumina BeadExpress instrument. The analyzer is currently being used to generate genomic data on 12 different projects. These projects are expected to generate preliminary data that will be published and will also allow the university to compete for additional grant funding based upon the proof of principle demonstrated in each experiment. Finally, because of the presence of the Illumina Genome Analyzer on the MU campus, acquired by these funds, Dr. Taylor was able to generate sufficient preliminary data to lead the development of a $5 million grant in the genomics of feed efficiency in beef cattle and was invited to participate in a second successfully funded $9.75 million grant led by Texas A&M University to study the genetics of resistance to bovine respiratory disease.

Quantitative Proteomics

Drs. John Walker, Jay Thelan, Scott Peck (UMC Project 00018355)

The instruments purchased using MLSRB funds have enhanced the state-of-the-art Charles W Gehrke Proteomics Center at MU. These instrument purchases although funded in bulk by the MLSRB also leveraged more than $370,000 in additional funds from the MU campus.

The research conducted by grant-supported postdoctoral fellows resulted in three publications and in addition to these published works three other manuscripts are in preparation. These manuscripts not only come directly from the PIs' labs but also represent research across campus, specifically in the Vet School and Biological Sciences, that was facilitated by the instruments purchased.

The infrastructure put in place by the quantitative proteomics MLSRB grant has also allowed award of over $1.7 million in National Science Foundation funding to members of the quantitative proteomics team and the proteomics center and, in part, attracted a $7.5 million National Institutes of Health grant to fund a new Botanical Center at MU.

It is impressive not only that the MLSRB investment has resulted in new federal money to MU, scholarly works, and leveraging of local funds, but that the infrastructure now in place will facilitate new research that will benefit the state of Missouri in the long term.

Water Quality

Dr. John Jones (UMD Project 00018356)

Streams draining 95 north Missouri watersheds were sampled in 2008 and 2009 to investigate the influence of confined animal feeding operations (CAFO's) on stream nutrients and related water quality variables during periods of baseflow when streams are fed by groundwater and are relatively free from effects of surface runoff. Watersheds were chosen to include a wide range land uses and potential CAFO effects as measured by the number of CAFO animal units in and near each watershed and the amount of land comprised by CAFO facilities and waste-application fields. Effects of CAFO's versus effects of non-CAFO influences were assessed by comparative analytical methods including correlation and multiple regression. Simple correlations revealed no strong relations between CAFO indices and any of 28 water quality variables measured. All but a few variables, however, were moderately to strongly correlated with general landscape features, including stream gradient and the proportions of cropland, grassland and forest in the watershed. Multiple regressions revealed weak, but statistically significant, secondary or tertiary effects of one or more CAFO indices on six of 28 water quality variables including potassium, nitrate-nitrogen and non-volatile suspended solids. Of these statistical effects, however, only the potassium relationship seemed to represent a relatively unequivocal example of a CAFO influence. This study, there, provides no evidence of a widespread influence of CAFO operations on phosphorus, nitrogen or organic matter during baseflow periods in north Missouri streams. CAFO effects seem more likely during high flow periods when surface runoff dominates stream conditions.

Odor Abatement

Dr. Monty Kerley (UMC Project 00018357)

Previous research conducted in our laboratory demonstrated the possibility of removing roughage from the diets of grain-fed cattle. This research demonstrated the potential to formulate diets that increased digestibility of the diet, primarily by removing the most indigestible ingredient (roughage or forage). By increasing diet digestibility and optimizing nutrient ratios relative to requirement by the animal, feed intake was reduced with no effect or improved growth performance. The result was cost effective diet changes that reduced manure volume in the growth phase by 60% and in the finishing phase by 40%. Methane production would have been at least a linear decrease response as well. We are planning on modeling this response with Canadian developed equations to determine the carbon trading value.

Dairy

Dr. Robert Kallenbach (UMC Project 00018358)

This grant mainly provided the milking and forage sampling equipment to conduct pasture-based dairy research. As a result of the technology purchased and developed by this grant, we have the capacity to conduct detailed evaluations of how daily fluctuations in pasture availability and nutritive value impact milk production. To our knowledge, MU has the only pasture-based dairy operation in the U.S. that can provide information on how variations in pasture change day-to-day milk yields.

Beef Herd

Dr. Monty Kerley (UMC Project 00018359)

Previous research we conducted had shown that 40% differences in intake can occur within a population of calves without any change in growth rate. Consequently, genetic potential exists that allows feed costs to be reduced potentially by 40%. The research sponsored by this grant allowed us to determine the effect of selection for this trait and the importance of formulating diets for efficiency phenotype. First generation progeny improved feed efficiency by 10 to 14% compared to progeny where efficiency was not used as selection criteria. We also determined that phenotype measured as a heifer was predictive of their offspring efficiency phenotype. We demonstrated that nutrient density needed to be changed to maximize growth performance depending upon efficiency phenotype. This research has the potential to demonstrate technology that can feed costs by 20% or greater.

Feed Mill

Dr. Rodney Geisert (UMC Project 00018360)

All bins are up and finalizing work to make them all functional. All items will be completed by 12/31/10.

Biomass

Dr. Jinglu Tan (UMC Project 00018361)

The project has been divided into three sub-projects: (1) Biomass production (Gene Stevens); (2) Biomass handling and process modeling (Jinglu Tan); and (3) Biomass conversion (Bill Jacoby).

Biomass production:

We have been studying sweet sorghum as a biomass crop. The latest results indicated that sweet sorghum is more efficient in producing maximum yields from nitrogen than corn. Harvesting sweet sorghum with a silage cutter would remove more nutrients than a sugar cane harvester. However, since most of the P and K are in the juice there was not as much difference as we expected. If the ethanol producing facility is close to production fields, growers should consider returning the vinasse material to the field after juice fermentation and distillation to replenish the soil with nutrients. The least amount of P, and K was removed from October and November switchgrass harvests, but growers need to access the risk of rainfall as the fall progresses in Missouri.

Biomass handling and processing modeling:

Based on the logistic model we developed, we performed more simulations to determine the best configurations for a processing plant like Show Me Energy in terms of optimal plant capacity, material mix, transportation methods and other parameters. The results showed that the plant capacity could be increased several fold before transportation distance negates profitability and net energy output. We have also been analyzing the mechanical properties of biomass in order to devise an efficient size-reduction mechanism. The data indicate that moisture plays a varied role and that grinding motion affects efficiency.

We have begun to develop a model to describe and simulate the dynamic behavior of a biomass supply chain. The model system is the MU Power Plant, which targets to increase its biomass usage to 25%. Data collection is under way. We have sought additional funding to continue this work after the LSTF project ends.

Biomass conversion process:

We have been studying extraction of triglycerides using supercritical carbon dioxide. We performed a detailed comparison this process with the conventional hexane crush process used in industry. We found advantages with respect to pretreatment (de-hulling not necessary) and separations (high-pressure equilibrium phase separation is very efficient from an energy standpoint and produces a clean oil that

does not require de-gumming). We also showed that the process is much faster and more thorough that previously reported. These factors led to a superior energy balance relative to the HCP.

We have also investigated gasification of biomass in supercritical water. As above, we compare it to conventional gasification processes in air and/or steam. We have shown the SCWG efficiently converts a wide variety of biomass to a similar product mixture containing hydrogen, methane, carbon dioxide and smaller amounts of ethane and carbon monoxide (Venkitasamy 2010). Further, we have shown that SCWG is very fast (six times faster than previous reports) and mates extremely well with high-pressure equilibrium separators to produce a clean, dry, high-value fuel gas.

Viticulture

Dr. Keith Striegler (UMC Project 00018362)

Nematode Survey:

In the fall of 2008, a comprehensive survey of nematode population density was conducted in commercial vineyards. One hundred seven samples were collected from selected vineyards which represented the primary grape production regions of Missouri and Arkansas, and submitted to a nematology laboratory for population density analysis down to species level. Particular emphasis was placed upon the Xiphinema americanum-group nematodes which are known to be important vectors of grapevine nepoviruses. Ninety eight percent of the submitted samples contained populations of X. americanum-group nematodes, demonstrating their widespread distribution across the region, in multiple soil types and on multiple host species. Pin, ring, and spiral nematodes were also detected at significant population densities in certain locations.

Viticultural Performance of Vines Expressing Symptoms of Virus Infection:

Experiments were established on own-rooted 'Chardonel' grapevines in Boone County and 'Cabernet Sauvignon' vines in St. Charles County, Missouri in 2008. The host vineyards are commercial blocks that have previously demonstrated symptoms of virus infection in portions of the vine population. Vines for study were selected based upon their distribution within the vineyard block and either presence or absence of visual virus symptoms. Populations of both symptomatic and asymptomatic vines were selected. Data collected to date have included grown pruning weight and shoot numbers, yield and yield components, and fruit composition. 'Chardonel' vines displaying symptoms of virus infection have generally displayed reduced vine size and yields, and vine mortality has been documented in the symptomatic population. As a consequence of our efforts to identify the virus/virus complex causing these symptoms, we have collaborated with scientists at Missouri State University and Foundation Plant Services at UC-Davis to analyze tissue samples for virus presence. Leafroll-associated viruses, tomato ringspot virus, grapevine fanleaf virus and rupestris stem pitting virus were identified in a large number of samples from symptomatic vines.

In part from our experiences with these experiments, we collaborated on an unsuccessful SCRI proposal in 2009, intended to expand this research to a greater depth and breadth. We intend to submit an AFRI proposal in 2011 based upon the initial research results noted above.

FY2008 Funding Summary – Commercialization

Grant Number	Project Title	Principal Investigator	PI Institution	Grant Category	Funds Awarded
13319	Polyhydroxyalkanoates in Transgenic Oilseeds	Dr. Jan Jaworski	Danforth Plant Science Center	Plant Science	$1,136,719
13320	Animal Waste Phosphorous Management Systems	Gary Clapp and Bill Junk	Institute for Industrial and Applied Life Sciences	Odor Abatement, Water Quality, Bioenergy	$325,000
13323	Commercialization of Value-Added Food-Grade Soybean Lines Developed by the University of Missouri and New Generation Functional Food Ingredients and Plant-Made Component for Nutritional Retail Products	Alex Stemme and Dr. Henry Nguyen	Mid-America R&D Foundation	Gateway	$738,281
13324	Commercialization of a Proprietary Bull Fertility Test	Dr. Peter Sutovsky	University of Missouri-Columbia	Animal Health	$400,000
				TOTAL	**$2,600,000**

FY2008 Commercialization Project Summaries

Project #:	13319-2007
Project Title:	Polyhydroxyalkanoates in Transgenic Oilseeds
Award Amount:	$1,136,719
Center for Excellence:	St. Louis
Lead Investigator:	Dr. Jan Jaworski, Donald Danforth Plant Science Center
Collaborators:	Edgar Cahoon and Joseph Jez, Donald Danforth Plant Science Center

Summary:
Polyhydroxyalkanoates (PHA) are aliphatic polyesters accumulated in certain bacteria as storage materials, but their physical properties are suitable for industrial uses as a renewable source of biodegradable thermoplastics. This project is a collaboration between Danforth Center researchers and scientists at Metabolix (www.metabolix.com) for the production of PHA in plants. The proposed work was to develop the technological foundation for efficiently producing these biomaterials in non-food crops and to create of a new Metabolix group in St. Louis. Our research objectives were to 1) produce transgenic *Brassica juncea* (Indian mustard) and *Camelina sativa* (False flax) with seed-specific expression of genes for the production of PHA. The goal was to have plants with seeds containing 5% dry weight PHA within 3 years and reduce to practice the production of a PHA-based plastic within 5 years; and 2) initiate studies for the second generation of plants producing maximal levels of PHA for commercialization.

Update:
The production of PHB in *Brassica juncea* and *Camelina sativa* progressed very well, although not without surprises. Synthesis of PHB requires three genes encoding 3-ketothiolase (BktB), acetoacetyl-CoA reductase (PhbB), and PHB synthase (PhbC). These were successfully cloned into appropriate

vectors for production of PHB targeted to seeds. Transformation of camelina led to seeds with as much as 10-15% PHB, which exceeded our goal by 2-3 fold. This clearly demonstrated that these transgenic seeds have the capacity to produce PHB in excess of 10%. Unfortunately these seeds could not germinate to produce the subsequent generations of plants. This required a major shift in our strategy, and in collaboration with scientists at Metabolix, Inc, we devised four different strategies to overcome this problem. We pursued all four strategies simultaneously, with two strategies pursued at the Danforth Center and two pursued by Metabolix scientists. All four strategies revolve around the use of an inducible promoter which would allow us to turn genes on or off by the addition of inducer. By the end of the funding period, we had successfully produced plants using all four strategies. Unfortunately, technical difficulties with regulation of the inducible promoter did not permit us to properly evaluate the effectiveness of each strategy. Overall, the project successfully demonstrated the feasibility of producing PHB in plant seeds and future work would focus on the temporal regulation of that production.

Project #:	13320-2007
Project Title:	Animal Waste Phosphorous Management Systems
Award Amount:	$325,000
Center for Excellence:	Kansas City
Lead Investigator:	Dr. Gary Clapp, Institute for Industrial and Applied Life Sciences and Bill Junk, DT Search & Designs, LLC
Collaborators:	Dean Thompson, DT Search & Designs, LLC and Gina Becker, Advanced Manufacturing Institute; Sigifredo Castro, Advanced Manufacturing Institute; Bret Lanz, Advanced Manufacturing Institute; Kylo Heller, KLA Environmental Services, Inc;.Frank Mercurio, KLA Environmental Services, Inc.; and Rick McKee, Kansas Environmental Management Associates

Summary:
The project addressed the issue of phosphorus imbalances at Confined Animal Feeding Operations (CAFOs) in Missouri and the Midwest. A phosphorus imbalance occurs when there is more phosphorus entering the operation (via animal feed) than leaving the operation (via harvested crops and animals for market). The result is excess phosphorus buildup in soils where manure is applied, which leads to a higher potential for phosphorus to be washed into surface water, negatively impacting water quality. In an effort to protect water quality, the Environmental Protection Agency (EPA) recently promulgated regulations requiring all CAFOs to balance the phosphorus in the waste they apply to crop fields with the phosphorus removed by the subsequent crop harvest.

Without the technology developed as part of this project, the only solution for CAFOs that had a phosphorus imbalance was to purchase more land on which to spread their manure, thereby distributing a smaller amount of manure on each acre of land. Often, this solution is not economical, and in some areas where land availability is limited, it is not even available. As a result, all Missouri and Midwest producers are faced with federal regulatory requirements and limited options for compliance.

To address this issue, DT Search and Designs, along with their collaborators, developed a waste treatment system to reduce the amount of phosphorus in the manure allowing CAFOs to apply more manure per acre while complying with regulatory requirements. The system also sequesters the removed phosphorus in a form that is easy to handle, transport, and process. The removed phosphorus has several unique characteristics that make it a valuable fertilizer that can be hauled to phosphorus deficient areas.
The goals of the project are listed below:

1) Design and develop the full-scale phosphorus management system,
2) Construct the full-scale unit, and
3) Complete system start-up and monitoring.

The site selection process included all of the likely CAFOs in the state of Missouri and the surrounding states. Supreme Cattle Feeders, LLC in Seward County, Kansas was chosen as the location for the farm-scale trial. The location was chosen for several critical reasons including the existing size of the CAFO, the ability to process water from the current waste storage system configuration, the historical wastewater volume, and the cooperative nature of the feedyard management. A similar configuration in the state of Missouri could not be located when the system was ready for deployment/testing.

The design of the farm-scale phosphorus reduction system was completed in the last quarter of 2007, by our joint venture with KEMA and the Advanced Manufacturing Institute (AMI) of Kansas State University (KSU). Primary design objectives included maximizing automation and thereby minimizing operating labor; making the system robust and simple enough to withstand substantial use and harsh weather with minimal maintenance; and maximizing efficiency while minimizing cost. These objectives were accomplished, and multiple companies were contacted to bid on system components.

Construction of the farm-scale unit began with the concrete being poured on April 18, 2008, and allowed to cure greater than 30 days to ensure design strength was reached. The farm-scale reactor and catwalk was delivered and installed on May 30-31, 2008 (see Figure 1). Bids for the reactor were evaluated and the lowest and best bid was determined to be Palmer. The reactor and catwalk were then built by Palmer Manufacturing & Tank Inc. The pipe fitting and electrical work was performed by local/regional contractors, and began soon after the reactor installation. Both were completed within a few weeks.

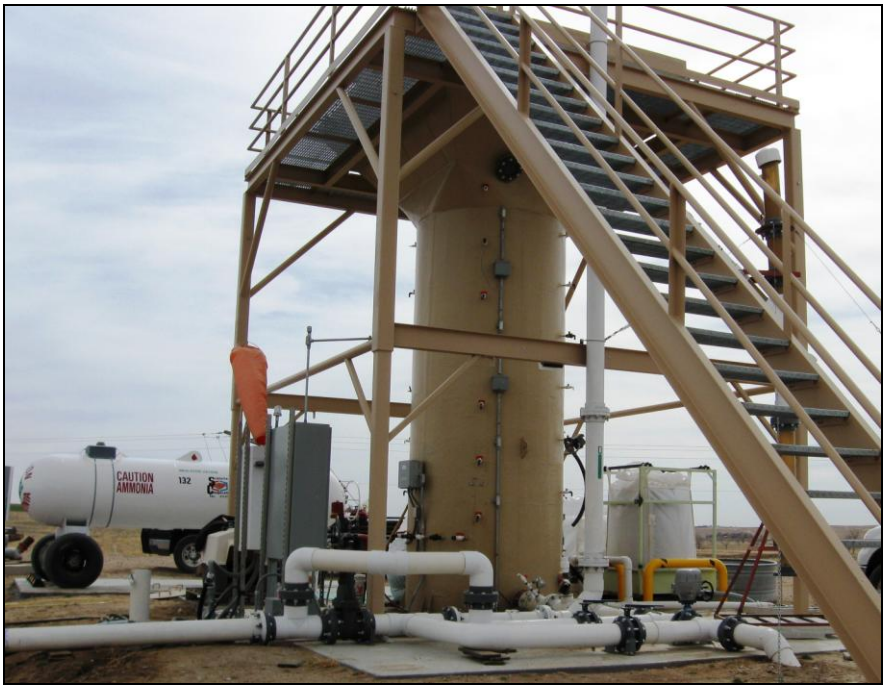


Figure 1 - Installed Phosphorus Reduction System

Start-up and monitoring began immediately following construction completion. However, unanticipated wastewater problems slowed the start-up process. Wastewater volumes at beef feedyards are primarily dependent on rainfall. Typically, this feedyard deals with a large volume of wastewater each year. Unfortunately, the winter, spring, and early summer of 2008 were abnormally dry, and therefore, the wastewater available was minimal. The wastewater that was available was of poor quality, i.e. non-typical wastewater characteristics. Specifically, it had extremely low nutrient levels. This substantially delayed the start-up process, and efforts to alter the wastewater quality to allow start-up and testing were not successful. However, the feedyard received substantial rainfall in August, and again in October. As

expected, the wastewater produced by the rain had typical characteristics for feedyard wastewater, and thus was ideal for start-up and testing

The primary objective of the start-up process was to create enough phosphate solids in the reactor for the fluidized bed to function properly. This proved more challenging than expected, and ultimately "seed material" was added to serve as reaction sites for phosphate solids growth. This approach was successful, and soon adequate phosphate solids had been produced for the fluidized bed to function properly. At that point, efforts shifted from start-up to monitoring.

Figure 2 - Phosphate Solids By-product

The primary objectives of the monitoring phase were to document phosphorus removal efficiencies under different operating conditions and to determine proper system operating procedures. Testing showed phosphorus removal rates as high as 63%, with most initial trials showing a phosphorus removal of approximately 50%. These removal efficiencies indicate the system is functioning successfully and correctly. To put this in context, a 50% reduction of the wastewater phosphorus would decrease the required amount of land for waste application by half, resulting in a substantial cost savings for the feeding operation. However, some inconsistency was seen. During the rest of 2009, multiple configurations and amendments were made to the process, yet none were able to achieve enhanced results over previous runs.

At times, monitoring indicated phosphorus removal rates of 30% or less which is below optimal levels. This appears to be an ammonia distribution issue. System modifications to eliminate such inconsistencies are part of ongoing optimization efforts being performed simultaneously, while preparations are being made for system commercialization. More than 30 million gallons of wastewater has been processed and approximately 13 tons of phosphate solids by-product (see Figure 2) has been produced.

During most of 2009, proper system operating procedures were continually investigated and refined during the monitoring phase, and a User's Manual was developed to document those procedures and represented much of the 2009 activities. The User's Manual will be a valuable resource for standard operation, as well as troubleshooting and maintenance.

The long-term benefits for Missouri and the region of this project can be separated into two main categories: 1) enhancement of natural resources and 2) sustainable operation and growth of agricultural businesses. The project will serve to enhance natural resources, primarily surface water. The technology allows surplus phosphorus to be easily moved to areas where there is a phosphorus deficit. Elevated phosphorus levels in surface water can cause algae blooms, fish kills, and foul odors. Removing excess manure phosphorus before it is applied to crop fields, effectively limits the amount of phosphorus available to be washed off of the field into surface water. Furthermore, the removed phosphorus can be taken to phosphorus-deficient areas and used to enhance plant growth decreasing the need for synthetic phosphorus fertilizers. The removed phosphorus is captured in an insoluble form which results in a slow release of nutrients when the material is applied to soil or other growth media. The slow release nature of the removed phosphorus serves to further protect surface water quality by limiting the amount of phosphorus that could be washed into surface water at any particular time.

Faced with financially burdensome regulations, the sustainable operation and growth of agricultural businesses is made possible by this technology. This technology will allow Missouri and regional CAFOs with limited options to achieve regulatory compliance in a fiscally responsible manner and could be the difference between staying in business and being forced to cease operations. Agricultural operations are the backbone of most rural Midwest and Missouri areas. Their commerce impacts other rural businesses and is critical to the long-term economic survival of nonurban areas.

Statement of Results:

In summary, we are pleased with the 2008 and 2009 project results. DT Search and Designs, through its joint venture, has found that the system can remove waste phosphorus by up to 63% and that it can be simple, robust, and easy to operate. One of the most surprising findings was the variability of the wastewater encountered at CAFOs. Extreme variations in pH, nitrogen, phosphorus, calcium, magnesium, bicarbonate, and other constituents indicated that phosphorus removal results would vary based on the waste characteristics. Uncontrollable wastewater characteristics further emphasized the need for the system to be robust. Optimizing ammonia distribution for pH adjustment in order to maximize uniformity was one of the lessons learned. This optimization makes the system more robust in order to manage wastewater quality changes. The varying levels of suspended solids in the waste also created an unforeseen need for a rotating screen on the system pump inlet, and thus it was learned that this simple addition could make the system more robust for handling changing waste solids concentrations.

Measurement and control of pH was expected to be rather straight forward, but was found to be quite complex. System modifications were made to consider temperature in the pH control mechanism. Another lesson learned was the ammonia delivery mechanism was found to be inadequate during cold weather, and thus a modified delivery system was installed.

The collaborators remain enthusiastic about the potential for this technology to help Confined Animal Feeding Operations improve the environment, increase operating efficiency, and achieve regulatory compliance. To do so, the collaborators are moving forward as quickly as possible with the commercialization of this technology. We fully anticipate this technology to be available to the Missouri CAFOs, as well as those throughout our region in the very near future.

Project #:	13323-2007
Project Title:	Commercialization of Value-Added Food-Grade Soybean Lines Developed by the University of Missouri and New Generation Functional Food Ingredients and Plant-Made Component for Nutritional Retail Products
Award Amount:	$738,281
Center for Excellence:	Statewide
Lead Investigator:	Alex Stemme, Mid-America Research and Development Foundation, Jefferson City; Dr. Henry T. Nguyen, Director at the National Center for Soybean Biotechnology, University of Missouri-Columbia
Collaborators:	W. J. (Bill) Cook, Missouri Food and Fiber; Ryan Schmidt, Soy Labs, LLC; David A. Sleper, National Center for Soybean Biotechnology; J. Grover Shannon, Delta Research Center, UM; and Richard J. Hofen, University of Missouri-Columbia

Summary:
This project seeks to commercialize several lines of technologically-enhanced soybeans developed by University of Missouri research scientists. These new soybeans have unique and special characteristics such as higher levels of oil and protein, or larger quantities of certain soy peptides demanded by the nutrition industry. These proprietary soybean varieties – grown in Missouri farmers' fields – will then be processed in Missouri into novel "functional food" ingredients (e.g. foods with benefits beyond basic nutrition).

This project will establish Missouri as the nation's leading intersection of plant science research and heart-healthy soy protein and functional food products – escalating the success of the National Center for Soybean Biotechnology at the University of Missouri. The project also assists in creating and developing a new commercial research institution, AgBorn Genetics, LLC. It will also attract and further develop the commercial firm, Soy Labs, LLC. Ultimately, the project will deliver new, Missouri-founded plant science technologies to the global marketplace in the form of functional food ingredients.

Update:
Due to its unique chemical composition, soybean seed is a very valuable and useful agricultural commodity. Among legumes and cereals it has the highest protein content (40%) and the second highest oil content (20%). Soybean is the world's primary source of plant protein and oil for humans and an important source of protein feed for livestock.

Soybean consumption may reduce cancer, blood cholesterol, osteoporosis and heart disease in humans, and soy contains minerals, vitamin B, folic acid and isoflavones purported to inhibit cancer, heart disease and osteoporosis. Global demand for food-grade soybean for human consumption has increased, primarily due to soy's perceived health benefits and nutritional value. Thus, there is increased economic potential and need for identification and development of value-added, high-yielding, food-grade soybean varieties.

We have identified exotic soybean germplasm for desirable concentration of bioactive compounds such as isoflavones and lunasin and seed composition traits such as protein and oil and to develop elite germplasm and varieties adapted to the northern and southern soybean production regions in Missouri. We have screened around hundreds of soybean germplasm and several mapping population to study the genetic differences in these compounds.

The project identified soybean lines higher in total isoflavone content and higher in genistein content (genestein is one of the major isoflavone components with the anticancer properties). Normal distribution of isoflavone and saponin seed contents in a preliminary experiment and QTL mapping reflects the additive effects of several genetic loci throughout the soybean genome.

We have identified soybean lines higher in the bioactive peptide, lunasin content. Oil and protein content analysis of soybean germplasm has identified plant introduction with higher protein content and screening of more PIs and mutant population. We have grown soybean lines selected for various seed compositional traits such as oil content and composition, protein content, lunasin content, isoflavone content at various environmental conditions and locations to study the stability of these compounds across the environments.

Statement of Results:

This project was successful in helping to recruit Soy Labs to the Missouri Plant Science Research Center in Mexico, Missouri – which is in the final stages of development and implementation – catapulting Missouri as the center of plant science research and the soy in human health marketplace. This project led to the production of soybeans from Missouri fields harvested this fall, which should be then processed in Missouri as soon as equipment is installed and the construction is completed at the new center.

This project will allow the nutraceutical and functional food company, Soy Labs, to grow their Lunasin XP® enriched soybeans from University of Missouri research scientists on Missouri farms and then process those specialty soybean lines (exhibiting powerful cholesterol-lowering properties) at the Missouri Plant Science Research Center in Mexico, Missouri. The resulting functional food ingredients will then be marketed to the nutrition industry throughout North America and to international markets from Europe to Southeast Asia.

Project #:	13324-2007
Project Title:	Commercialization of a Proprietary Bull Fertility Test
Award Amount:	$400,000
Center for Excellence:	Statewide
Lead Investigator:	Dr. Peter Sutovsky, University of Missouri-Columbia
Collaborators:	Dr. David Patterson, University of Missouri-Columbia

Summary:

This proposal seeks to commercialize a new, patented bull fertility test that will improve reproductive health and performance of dairy and beef bulls, thus adding to the bottom lines of many Missouri dairy and beef cattle producers. Current fertility evaluation in bulls is based on subjective methods introduced in the 1950's. The test that is the subject of this grant represents an accurate, inexpensive, and commercialized viable approach to improving efficiency in this area. This method centers on detection of sperm surface molecules that are found only in defective sperm cells, which provides a quick evaluation of bull fertility and diagnosis of reproductive disorders. The goal of this project is to develop and commercialize the following products for fertility testing in bulls: **AIM 1**) Veterinarian's office bull fertility test kit; **AIM 2**) Reference laboratory service for bull fertility testing; and, **AIM 3**) Nanotechnology based semen purification kit to be used by artificial insemination companies. A start-up company spun off by the University of Missouri, or licensing to a Missouri-based animal health/artificial insemination company will be pursued as avenues for commercialization.

Update:

AIM 1: Veterinarian's office bull fertility test. The original research procedure for the detection of ubiquitin-tagged defective sperm incorporated laborious fixation (40 min), blocking (25 min), two consecutive incubations with primary and secondary antibodies, and multiple washes by centrifugation in between, altogether amounting to approximately three hours of processing and incubation. In the course of the present project, we have developed a very simple procedure for defective sperm tagging that only takes 20 minutes and does not involve any fixation, washing or centrifugation. This procedure has been developed for the labeling and evaluation of bull sperm cells (Fig. 1 A), but can be adapted for other farm animals (e.g. boars) as well as for humans (male infertility diagnostics). Briefly, one microliter of raw or frozen/thawed bull semen is mixed in a plastic Eppendorf tube with 100 microliters of labeling solution containing a commercially sourced anti-ubiquitin antibody (catalog # MK12-3, sourced from MBL International, Seattle, WA). After ten minutes, a second probe is added, which is a widely available fluorescently tagged secondary antibody that binds specifically to sperm-bound MK12-3 probe. After another ten minutes, a small amount (10 microliters) of the sample is mounted on a microscopy slide and one hundred spermatozoa are scored for the presence of fluorescently tagged ubiquitin. Fertile bulls are expected to have no more than 25% tagged spermatozoa.

AIM 2: Reference laboratory service for bull fertility testing. The original intent of this Aim was to develop a test that would be used in a reference laboratory receiving test semen samples from AI companies and individual farming operations. Though profitable, this strategy would have limited the dissemination and commercial success of this technology to a single testing facility. An unexpected opportunity to adapt this test for worldwide distribution with a dedicated instrument for automated semen analysis arose following Dr. Sutovsky's presentation of this project's results at the technical Conference of the National Association of Animal Breeders in Milwaukee, 2008. At this conference, IMV Technologies, Maple Grove, MN, introduced a simple, relatively inexpensive, dedicated sperm flow cytometer Guava EasyCyte Plus (**Fig. 1 B**), marketed to AI companies for routine bull fertility testing. Following this meeting, Dr. Sutovsky started a collaboration with IMV to add the UBI-test to the lineup of sperm tests on the Guava Instrument. IMV provided one such instrument free of charge to Dr. Sutovsky's lab. As a result of technology development and validation in our lab, IMV licensed a UBI-test and a complementary PAWP test (see Fig. 1 A). The same 20 min procedure for UBI-labeling, described in AIM1, has been adapted for Guava. A very similar dual UBI-PAWP labeling procedure (see **Fig. 1 A**) has also been developed for this instrument. IMV is the world leader in supplies and equipment for artificial insemination (AI), having 75% of the worldwide share in cattle AI and 35% in the swine AI industry. A large scale trial has been completed on 160 bulls with a goal of validating the ubiquitin test on the Guava platform. Strong negative correlations were found between ubiquitin and conventional parameters of semen quality. A manuscript has been prepared detailing these results, pending submission to a scientific journal.

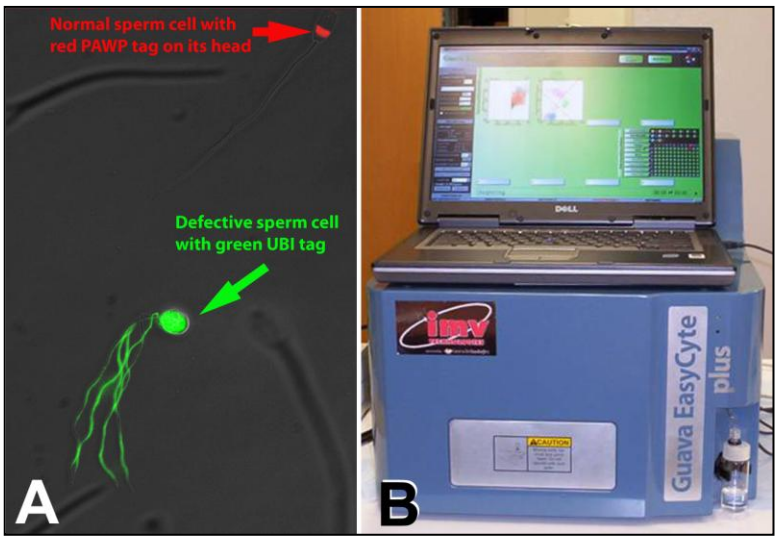


FIGURE 1: (**A**) Dual detection of ubiquitin (green) and PAWP protein (red) in bull semen. Normal sperm cells show PAWP tag on their posterior head; defective sperm are tagged with ubiquitin on both head and tail. (**B**) Dedicated sperm cytometer Guava EasyCyte Plus, marketed by IMV Technologies. IMV licensed our UBI and PAWP sperm tests to be included in Guava software.

AIM 3: Nanotechnology based semen purification kit for AI companies. Ubiquitin-binding nanoparticles for the removal of defective sperm cells from bull semen have been developed and tested successfully in bull sperm depletion trials. This simple procedure does not require any extensive semen handling/centrifugation and involves only a 15 minute incubation of semen with nanoparticles that bind exclusively to defective sperm cells, followed by a 15 minute magnetic depletion time and skimming of fertile sperm fraction. A manuscript detailing this technique has been written, pending final editing and submission. Measurements of sperm viability by flow cytometry demonstrated a statistically significant improvement in the viability (percentage of live/viable sperm cells) in semen samples depleted by our nanoparticles. Enrichment of defective sperm cells has been documented in the nanoparticle fraction discarded after depletion. Most importantly, tests by in vitro fertilization (IVF) demonstrated a statistically significant improvement in the overall fertilization rate using sperm fractions depleted with UBI-binding nanoparticles, compared to fractions mock-depleted with control, inactive nanoparticles.

The light microscopic ubiquitin-based sperm evaluation procedure has been tested by veterinarians at ABS Global, DeForest, WI, a major US artificial insemination (AI) company. Flow cytometric version is currently being tested by Genex Inc, who provided samples as well as matching funds for this work. The Guava Instrument is already on market and both UBI and PAWP assays have been licensed to IMV Technologies. Additional licensing deals will be negotiated during the visit of IMV's director of R&D, Dr. Eric Schmitt, to MU, scheduled for February 7-8, 2011.

The functional prototype of the nanodepletion kit is a result of collaboration with Clemente Associates, Madison, CT, a biotech company specializing in magnetic purification of cultured cells. The intent is to market this product directly to end users in the AI industry, or to offer the semen depletion kit through cooperation with IMV Technologies. A secondary market is being explored for the use in human infertility clinics.

Present funding resulted in creating an equivalent of one new full time position, while two additional existing positions have been supported partially, and partial support was also provided for one graduate student/research assistant on this project. Additional job creation resulting from this project will most likely come from hiring staff to work for Dr. Sutovsky's startup, AndroLogika LLC, Columbia MO, which will specialize in kit assembly for Guava tests and/or fertility testing serving the cattle and pig AI industry.

MLSRB

MISSOURI LIFE SCIENCES RESEARCH BOARD

Annual Audit Report FY2010

COMMUNICATION OF AUDIT RESULTS

MISSOURI LIFE SCIENCES RESEARCH TRUST FUND

June 30, 2010

December 23, 2010

To the Board of Directors of
Missouri Life Sciences Research Trust Fund

We have audited the financial statements of Missouri Life Sciences Research Trust Fund for the year ended June 30, 2010, and have issued our report thereon dated December 23, 2010. Professional standards require that we provide you with the following information related to our audit.

Our Responsibility under U.S. Generally Accepted Auditing Standards

As stated in our engagement letter dated December 10, 2010 our responsibility, as described by professional standards, is to express an opinion about whether the financial statements prepared by management with your oversight are fairly presented, in all material respects, in conformity with the basis of cash receipts and disbursements, which is a comprehensive basis of accounting other than accounting principles generally accepted in the United States of America. Our responsibility is to plan and perform the audit to obtain reasonable, but not absolute, assurance that the financial statements are free of material misstatement. Our audit of the financial statements does not relieve you or management of your responsibilities.

As part of our audit, we considered the internal control of Missouri Life Sciences Research Trust Fund. Such considerations were solely for the purpose of determining our audit procedures and not to provide any assurance concerning such internal control. Our internal control findings and other recommendations are included in a separate communication of significant deficiencies letter.

Significant Accounting Policies

Management is responsible for the selection and use of appropriate accounting policies. In accordance with the terms of our engagement letter, we will advise management about the appropriateness of accounting policies and their application. The significant accounting policies used by Missouri Life Sciences Research Trust Fund are described in Note 1 to the financial statements. No new accounting policies were adopted and the application of existing policies was not changed during the fiscal year. We found no transactions entered into by the Fund during the year for which there is a lack of authoritative guidance or consensus.

Audit Adjustments

For the purposes for this letter, professional standards define an audit adjustment as a proposed correction of the financial statements that, in our judgment, may not have been detected except through our auditing procedures. An audit adjustment may or may not indicate matters that could have a significant effect on the Fund's financial reporting process (that is, cause future financial statements to be materially misstated).

We did not propose any audit adjustments for the year.

Accounting Estimates

Accounting estimates are an integral part of the financial statements prepared by management and are based on management's knowledge and experience about past and current events and assumptions about future events. Certain accounting estimates are particularly sensitive because of their significance to the financial statements and because of the possibility that future events affecting them may differ significantly from those expected.

Difficulties Encountered in Performing the Audit

We encountered no significant difficulties in dealing with management in performing and completing our audit. All of the Fund's personnel cooperated with us fully during our audit.

Disagreements with Management

For purposes of this letter, professional standards define a disagreement with management as a financial accounting, reporting, or auditing matter, whether or not resolved to our satisfaction, that could be significant to the financial statements or the auditor's report. We encountered no such disagreements with management during the course of our audit.

Management Representations

We have requested certain representations from management that are included in the management representation letter dated December 23, 2010.

Management Consultations with Other Independent Accountants

In some cases, management may decide to consult with other accountants about auditing and accounting matters, similar to obtaining a "second opinion" on certain situations. If a consultation involves application of an accounting principle to the Fund's financial statements or a determination of the type of auditor's opinion that may be expressed on

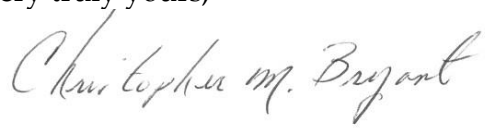
those statements, our professional standards require the consulting accountant to check with us to determine that the consultant has all the relevant facts. To our knowledge, there were no such consultations with other accountants.

Other Audit Findings or Issues

We generally discuss a variety of matters, including the application of accounting principles and auditing standards, with management each year prior to retention as the Fund's auditors. However, these discussions occurred in the normal course of our professional relationship and our responses were not a condition to our retention.

We wish to thank the Missouri Life Sciences Research Trust Fund personnel for their assistance during the course of our audit. We will be pleased to discuss these or any other matters at your convenience. This information is intended solely for the use of Board of Directors and management of Missouri Life Sciences Research Trust Fund and is not intended to be and should not be used by anyone other than these specified parties.

Very truly yours,



Christopher M. Bryant, CPA

MISSOURI LIFE SCIENCES RESEARCH TRUST FUND

JEFFERSON CITY, MISSOURI

INDEPENDENT AUDITORS' REPORT

For the Year Ended June 30, 2010

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INDEPENDENT AUDITORS' REPORT

To the Executive Board of
Missouri Life Sciences Research Trust Fund:

We have audited the accompanying financial statements of Missouri Life Sciences Research Trust Fund (a component of the State of Missouri), as of and for the year ended June 30, 2010, which collectively comprise the Fund's basic financial statements as listed in the table of contents. These basic financial statements are the responsibility of the Fund's management. Our responsibility is to express an opinion on these financial statements based on our audit.

We conducted our audit in accordance with auditing standards generally accepted in the United States of America. Those standards require that we plan and perform the audit to obtain reasonable assurance about whether the financial statements are free of material misstatement. An audit includes examining, on a test basis, evidence supporting the amounts and disclosures in the financial statements. An audit also includes assessing the accounting principles used and significant estimates made by management, as well as evaluating the overall financial statement presentation. We believe that our audit provides a reasonable basis for our opinion.

As discussed in Note 1, the Fund prepares it financial statements on the basis of cash receipts and disbursements, which is a comprehensive basis of accounting other than generally accepted in the United States of America.

In our opinion, the financial statements referred to above present fairly, in all material respects, the respective cash basis financial position of the fiduciary activities of the Missouri Life Sciences Research Trust Fund as of June 30, 2010, and the respective changes in cash basis financial position thereof for the year then ended in conformity with the basis of accounting described in Note 1.

Management has elected to omit the Management's Discussion and Analysis section from the audit report.

CHRISTOPHER M. BRYANT, CPA
St. Louis, Missouri

December 23, 2010

BASIC FINANCIAL STATEMENTS

The basic financial statements include integrated sets of financial Statements as required by GASB. The sets of statement include:

- Fund financial statements
  - Fiduciary Fund

In addition, the notes to the financial statements are included to provide information essential to the user's understanding of the basic financial statements.

	BEGINNING NET ASSETS	RECEIPTS	DISBURSEMENTS	ENDING NET ASSETS
ACTIVITIES FUND				
Life Science Research Trust	$ 4,631,794	$ 35,079,732	$ 38,366,349 *	$ 1,345,177
Total Activities Fund	$ 4,631,794	$ 35,079,732	$ 38,366,349	$ 1,345,177
NET ASSETS				
Cash				1,345,177
TOTAL NET ASSETS-CASH BASIS HELD IN TRUST				$ 1,345,177

*** - See Note 5**

See accompanying Notes to the Financial Statements.

NOTE 1 – NATURE OF OPERATIONS AND SUMMARY OF SIGNIFICANT ACCOUNTING POLICIES:

The accompanying financial statements include the transactions of all funds under the management and control of the Fund's Board. The Fund is included as a component unit of State of Missouri.
The Missouri Life Sciences Research Trust Fund (the Fund) was established by the Missouri General Assembly beginning in fiscal year 2007 and in perpetuity thereafter. Moneys in the Fund shall be used strategically, in cooperation with other governmental and not-for-profit entities, to enhance the capacity of the State of Missouri's ability to perform research to better serve the health and welfare of the residents of the State of Missouri as a center of life sciences research and development by building on the success of research institutions located in Missouri, creating in and attracting to Missouri new research and development institutions, commercializing the life sciences technologies developed by such institutions, and enhancing their capacity to carry out their respective missions. Its governing body consists of seven members who have general familiarity with the life sciences and current research trends and are appointed by the Governor with the advice and consent of the Senate.

The Fund may establish and is to oversee each "center for excellence for life sciences research", which may be located in the St. Louis, Kansas City, Springfield or Missouri Statewide area.

The Fund is to be held separate and apart from all other public moneys and funds of the State, including but not limited to the tobacco securitization settlement trust fund. The state treasurer shall deposit into the fund twenty-five percent of all moneys received from the master settlement agreement.

No more than ten percent of the moneys shall be used for the construction of physical facilities and further provided that in any fiscal year eighty percent of the moneys shall be appropriated to build research capacity at public and private not-for-profit institutions to promote life science technology transfer and technology commercialization. Of the money appropriated to build research capacity, twenty percent shall be appropriated to promote the development of research of tobacco-related illnesses.

A. Financial Reporting Entity

The Fund's financial reporting entity is comprised of the following:

Primary Government: Missouri Life Sciences Research Trust Fund

In determining the financial reporting entity, the Fund complies with the provisions of Governmental Accounting Standards Board Statement No. 14, The Financial Reporting Entity, as amended by GASB 39 *Determining Whether Certain Organizations Are Component Units*

B. Basis of Presentation

Government-Wide Financial Statements

The Statement of Net Assets and Statement of Activities display information about the reporting Government as a whole. They include all funds of the reporting entity except for fiduciary funds. The statements distinguish between governmental and business-type activities. Governmental activities generally are financed through taxes, intergovernmental revenues and other non-exchange revenues. Business-type activities are financed in whole or part by fees charged to external parties for goods or services. The Fund does not have any governmental or business-type activities. Therefore, no government-wide financial statements are included.

NOTE 1 – NATURE OF OPERATIONS AND SUMMARY OF SIGNIFICANT ACCOUNTING POLICIES: (Continued)

B. Basis of Presentation (continued)

Fund Financial Statements

Fund financial statements of the reporting entity are organized into funds, each of which is considered to be a separate accounting entity. Each fund is accounted for by providing a separate set of self-balancing accounts which constitute its assets, liabilities, fund equity, receipts, and disbursements. The fund is organized into one major category, fiduciary. The Fund presently has no governmental or proprietary funds. An emphasis is placed on major funds within the governmental categories. A fund is considered major if it is the primary operating fund of the Trust of meets the following criteria:

- Total assets, liabilities, receipts, or expenditures of that individual government fund are at least 10 percent of the corresponding total for all funds of that category or type, and

- Total asset, liabilities, receipts, or expenditures of the individual governmental fund are at least 5 percent of the corresponding total for all governmental funds combined.

The funds of the financial reporting entity are described below:

Fiduciary Fund

Private Purpose Trust Fund – Fiduciary funds are used to account for resources held for the benefit of parties outside the government.

C. Measurement Focus and Basis of Accounting

Measurement focus is a term used to describe "how" transactions are recorded within the various financial statements. Basis of accounting refers to "when" transactions are recorded regardless of the measurement focus applied.

Measurement Focus

In the fund financial statements, the "current financial resources" measurement focus or the "economic resources measurement focus," as applied to the cash basis of accounting, is used as appropriate:
All governmental funds utilize a "current financial resources" measurement focus. Only current financial assets and liabilities are generally included on their balance sheets. Their operating statements present sources and uses of available spendable financial resources at the end of the period.

Basis of Accounting

In the fund financial statements, fiduciary activities are presented using a cash basis of accounting. This basis recognizes assets, liabilities, net assets, receipts, and expenditures when they result from cash transactions. This basis is a comprehensive basis of accounting other than accounting principles generally accepted in the United States of America.

As a result of the use of cash basis of accounting, certain assets and their related receipts (such as accounts receivable and revenue for billed or provided services not yet collected) and certain liabilities

NOTE 1 – NATURE OF OPERATIONS AND SUMMARY OF SIGNIFICANT ACCOUNTING POLICIES: (Continued)

C. Measurement Focus and Basis of Accounting (continued)

and their related expenses (such as accounts payable and expenses for goods or services received but not yet paid) *are not recorded* in these financial statements.

D. Assets, Liabilities and Equity

Cash and Cash Equivalents
For the purpose of financial reporting "cash and cash equivalents" includes on demand and savings accounts, and certificates of deposit or short-term investments with original maturity of three-months or less.
Equity Classification
It is the Fund's policy to first use restricted net assets prior to the use of unrestricted net assets when an expense is incurred for purposes for which both restricted and unrestricted net assets are available.

Fund Financial Statements:
Fiduciary fund equity is classified as ending net assets.

NOTE 2 – DEPOSITS, INVESTMENTS AND INVESTMENT INCOME:

Deposits
Cash and cash equivalents are invested by the State as part of the State's cash pool. All deposit and investment risk is controlled by the State. Information concerning the State's deposit and investment risks may be found in the State's Comprehensive Annual Financial Report.

NOTE 3 – RISK MANAGEMENT:

The Fund is exposed to various risks of loss related to torts; theft of, damage to and destruction of assets; business interruptions; errors and omissions; natural disasters; employee injuries and illnesses; and employee health and accident benefits. Commercial insurance coverage is purchased for claims arising from such matters other than employee health benefits. Settled claims have not exceeded the commercial coverage in any of the three preceding years.

NOTE 4 – CONTINGENCIES:

Litigation – Various claims and lawsuits are possible against the Fund. In the opinion of the Fund management, the potential loss on all claims and lawsuits will not be significant to the Fund's financial statements.

NOTE 5 – REAPPROPRIATIONS:

The Missouri Life Science Research Board was informed in late October 2009 that the Trust Fund was included in the state spending restriction; and therefore, no grants were awarded in Fiscal Year 2010. During fiscal year 2010, money was transferred from Trust to Department of Social Services Pharmacy Program and Healthnet in the amount of $28,725,000 and $9,000,000, respectively.